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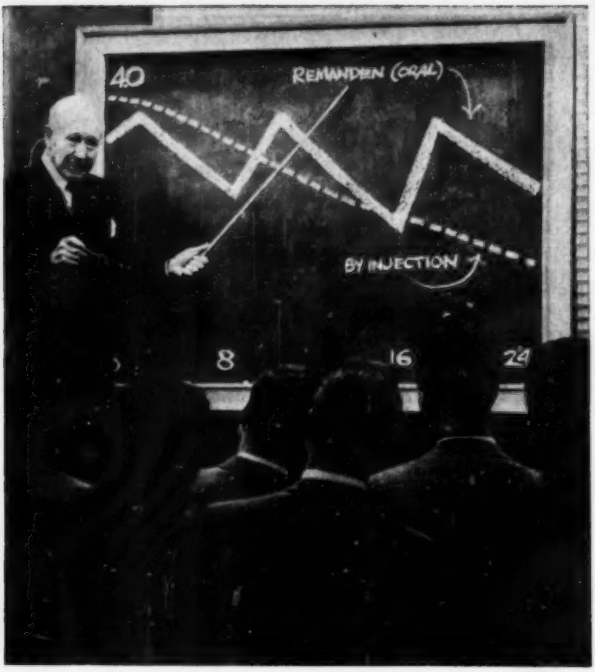

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CHARLES ROBERT AUSTRIAN
1885–1956

CHARLES ROBERT AUSTRIAN[1]

On the afternoon of July 13, 1956, The Johns Hopkins University lost one of its distinguished sons, and The Johns Hopkins Hospital one of its most distinguished staff members, when Dr. Charles Robert Austrian died suddenly in Baltimore of heart failure.

He had been vacationing with Mrs. Austrian in New York City and had interrupted that vacation in order to return to Baltimore to be present at an operation upon one of his patients. He was stricken while in an automobile outside the office of the surgeon who had been selected to perform the operation and, although he was taken to The Johns Hopkins Hospital as rapidly as possible, he died in the ambulance before he arrived there. Thus ended a brilliant career that was intimately associated with the two Hopkins institutions for more than a half century.

Austrian was born in Baltimore, Maryland, on May 28, 1885, the son of Robert and Belle (Bernei) Austrian. The father had established himself in the hat-manufacturing business but Charles was not destined to follow in his father's footsteps. He attended the public schools of the city and also a private school for boys, from which he entered The Johns Hopkins University in 1901 as a candidate for the A.B. degree.

He was an outstanding student, taking honors at graduation in 1904, and then entered the School of Medicine but almost immediately came down with an attack of typhoid fever which forced him to postpone the scheduled first year work for a whole year. He graduated from the School in 1909, again with honors, being first in his class, and in September of that year entered upon his duties as resident house officer in medicine in The Johns Hopkins Hospital. From that time until the day of his death he was an active member of the staff of that institution, and devoted much of his time and energy to it.

Following the completion of his internship he was appointed assistant resident physician in the Hospital, a post which he held until 1914. During three of those years he was in charge of the laboratory of the Phipps Tuberculosis Dispensary in addition to his regular duties as assistant resident physician.

In 1914 he resigned as a member of the resident staff of the Hospital and was appointed Associate in Medicine in the School. The records of the institution indicate that this was a full-time post and one of the first to be filled in a clinical department, for the year 1914–15 was the first year of operation of the full-time system in the clinical departments of the School. He retained this post for one year only. The call to enter the private practice of medicine

[1] Member of Editorial Board of Bulletin of the Johns Hopkins Hospital for twenty-six years.

appears to have been too strong to resist for in the summer of 1915 he opened his own office at 1417 Eutaw Place, an office which he never vacated until his death. He retained his connection with the Phipps Tuberculosis Dispensary, however, and in 1915 was placed in charge of this division of the Dispensary, a position which he held until 1917 when the Phipps was merged with the general medical clinic of the Dispensary.

Austrian's first appointment in the Medical Faculty was in 1910 as Assistant in Medicine and he remained an active member of the Medical Faculty until 1950, when he retired from his academic duties, having in the meantime been promoted through the grades of Instructor and Associate to that of Associate Professor. He never retired from active practice, however, and was still Visiting Physician to the Hospital when he died. He was also Physician-in-Chief of Sinai Hospital in Baltimore from 1921 to 1944.

Although Austrian's position in medicine will in the final analysis rest upon his superb skill as a clinician, he always had an abiding interest in the development of medical knowledge and contributed to that development, particularly in his earlier days. Indeed, while still a student, he found time to work with the late Walter Jones in the field of the nucleic acids, and formed an enduring friendship with that unique and engaging figure, but Austrian's chief interest came subsequently to be the field of the infectious diseases, and particularly that of tuberculosis. He acquired a high degree of skill in the diagnosis of diseases of the chest, perhaps due in part to his unusually acute sense of hearing, and in part also to his assiduous use of the X-ray film as an adjunct in chest diagnosis, but this skill must also have been due in no small measure to the many hours which he devoted to the examination of patients first in the Phipps dispensary and, later, in the chest clinic of the General Medical Dispensary. It is certainly no accident that that particular clinic should have been named after him several years ago.

It should be made clear, however, that Austrian is not to be thought of as a specialist in tuberculosis or in any other particular division of internal medicine, and he certainly did not wish to be so considered. His field was the entire human body, and it was the breadth of his interests and the depth of his knowledge, coupled with a sharp intellect, long experience and warm sympathy for the ailing human being that made him the superb doctor that he came ultimately to be. Small wonder that his colleagues sought his opinion when they or members of their family fell ill, or when they had patients whose symptoms baffled them completely. Austrian was in the direct line of the great clinicians that have graced the Johns Hopkins Hospital's Department of Medicine, and his name deserves always to be linked with those predecessors of his who set and maintained at the Hopkins the tradition of careful clinical observation in the study and care of patients.

Austrian's medical interests were not limited to the Hopkins, or to Sinai across the street, or to his own private practice. Like Osler, he took a lively interest in the affairs of the local medical profession. He was president of the Baltimore City Medical Society in 1938, president of the Medical and Chirurgical Faculty of Maryland five years later, and served as a member of that Faculty's council for thirty consecutive years. He was also a member of the Association of American Physicians, the American Climatological and Clinical Association, the Association of American Pathologists and Bacteriologists, and the Cosmopolitan Clinical Club, as well as the American Medical Association and the Southern Medical Association. In World War I he served as medical advisor to the State Director of the Selective Service System and also as a member of Medical Advisory Board No. 7 of the United States Army.

Nor were Austrian's interests by any means limited to the medical profession alone. He served his state for a while as a member of its Board of Welfare, was president and member of the Board of Trustees of Park School, a private school in Baltimore, and had served as a member of the Board of Trustees of the Peabody Institute for several years prior to his death.

In 1914 he married Miss Florence Hochschild and to them two children were born, a son and a daughter. The son has followed in his father's footsteps, and his successful career as a teacher and investigator in the field of medicine was a constant source of pride and pleasure to the father. So also were the achievements of the daughter in the field of economics prior to her marriage. The Austrian household has always been an exceedingly hospitable one and many a member of the resident staff of The Johns Hopkins Hospital has been priviledged to partake of that hospitality.

"Charley" Austrian's life was a rich one in every sense of the word. Able to devote many years to a training which fitted him to advance to the top of his profession, his skill, energy, knowledge based upon experience, and above all his wisdom, earned for him the respect and admiration of his colleagues, than which nothing is, or should be, more precious to any professional man.

The sudden occlusion of a coronary vessel in later life and the onset of gout forced him to curtail his activities appreciably but he would not use them as an excuse to give up practice altogether even though he would have been completely justified in doing so.

None of his friends ever heard him say a word about himself that could be interpreted as self-pity, even though they could see that at times he was in real pain. Nor did he ever betray any evidence that he was aware of the sword which hung over him during the last years of his life, although none knew better than he of the existence of that sword. Nor did he allow his awareness of that weapon to keep him last summer from thrusting aside his own ease

and pleasure in order to go to the aid of a patient many miles away, even though doing so involved an arduous journey which, as it turned out, was to be his last.

Austrian died as every medical man worth his salt wishes to die—swiftly and while actually discharging the duties of his calling. His manner of death was the epitome of his life, and those who cherished him, and they are many, would not have had it otherwise if they could.

ALAN M. CHESNEY

DISTURBANCE IN THE EXCRETION OF PARA-AMINOBENZOIC ACID METABOLITES IN LIVER DISEASE[1]

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While studies on the metabolism of sulfathiazole and sulfadiazine in man were in progress two of the subjects developed infectious hepatitis. During the course of the hepatitis in these two individuals it was observed that there were marked decreases of acetylated sulfonamide in the urine compared to the quantities previously excreted. *Pari passu* with recovery, excretion rates of the acetylated sulfonamide returned to the initial rates. These observations were extended using sulfamerazine, sulfisoxazole, and *p*-aminobenzoic acid, all appearing to confirm the initial observation. Bloomberg (1) and Gershberg and Kuhl (2) in their reports on human subjects with liver dysfunction concluded that there was no abnormality of acetylation in cirrhosis. Tabor *et al.* (3), however, showed in patients with liver dysfunction a diminished total excretion of *p*-aminobenzoic acid metabolites, a smaller proportion of *p*-aminohippuric acid, essentially unchanged absolute amount excreted of acetylated *p*-aminohippuric acid, and an elevated plasma level of *p*-aminobenzoic acid. The studies reported here confirm the observation of Tabor *et al.* that there is a diminished total excretion of metabolites of *p*-aminobenzoic acid. Evidence is presented that acetylation is reduced concomitantly with the total reduction of *p*-aminobenzoic acid metabolites and that these alterations reflect the state of hepato-cellular damage.

PROCEDURE

Based on the well established knowledge that *p*-aminobenzoic acid (PABA) when ingested undergoes extensive acetylation, is rapidly excreted in the urine, and is relatively non-toxic, PABA was used definitively in these studies rather than the sulfonamides. Riggs and Hegsted (4) have shown in the rat that the lower the dose of PABA the higher is its percentage acetylation. Gershberg and Kuhl (2) have shown the same to be true in man. In the present studies in man, one millimole (137 mg.) of crystalline PABA in a gelatin capsule was administered orally with water to a group of patients, in the fasting state when possible, of the Johns Hopkins and Baltimore City Hospitals. A control specimen of urine was collected under toluene before giving the drug. From the time of ingestion of the PABA a two-hour total urine collection was taken. Analyses for "free" and "total" PABA were made using the method of Bratton and Marshall (5) using a Klett colorimeter. Recoveries of PABA and *p*-aminohippuric acid (PAHA) added to the urine were quantitative. Both agents gave

[1] This study has been made possible by a Grant-in-Aid by the Hoffman-LaRoche Company, Nutley, N. J. Particular thanks are due Dr. W. A. Davis.

identical molar color development. There was no extraction of the measurable metabolites of PABA by toluene. The presence of jaundice did not affect the accuracy of the analyses. No ether extraction of the urine was made either before or after hydrolysis to separate the PABA and its derivative *p*-aminohippurate components (6). The acetylated fraction excreted was that calculated as the difference between the "free" and "total" values.

To ascertain that absorption of PABA from the intestinal tract in patients with liver disease was not sufficiently impaired to distort results, PABA (1 mM) was administered intravenously as well as orally to three individuals; one normal, one with portal cirrhosis, and one with biliary cirrhosis. The PABA for intravenous administration was neutralized with sodium hydroxide, autoclaved and injected in a volume of two millilitres (2 ml. = 1 mM PABA) over a 2–3 minute period. The time of urine collection was identical for both routes of administration. The data obtained, shown in Table I, indicates that there was an increase of about 20 per cent in acetylated PABA excreted after intravenous as compared with oral PABA. This increase occurred in all subjects. Since no time allowance in urine

TABLE I

Comparison of Urinary p-Amino Benzoic Acid Metabolites in Three Subjects Following the Administration of One Millimole of p-Amino Benzoic Acid

Diagnosis	Route of Administration	Urine* Volume (μm)	"Free" PABA (μm)	Per cent change with i.v. administration	Acetylated PABA (μm)	Per cent change with i.v. administration
(1) normal	oral	985	8		424	
normal	i.v.	100	143	+1688	515	+21
(2) portal cirrhosis	oral	34	31		295	
portal cirrhosis	i.v.	50	86	+177	346	+17
(3) biliary cirrhosis	oral	40	12		172	
biliary cirrhosis	i.v.	70	56	+367	207	+20

* 2 hour total beginning at time of PABA administration.

collection was made for the time of absorption by the oral route, an increase in excreted acetylated PABA by the intravenous route was anticipated. A demonstrable increase of the same magnitude was observed in all subjects. However, the increase in the "free" values differed widely in the three subjects and was many fold greater in each case than the observed change in the acetylated PABA values. It was felt, therefore, that the oral route of administration of the PABA in subjects with liver disease was acceptable.

RESULTS

As control values for excreted acetylated PABA, analyses for metabolites of PABA were made on two-hour urine collections from 12 healthy subjects following the administration of 1 mM PABA. These subjects comprised six men and six women, white and negro, ranging in age from 22 to 53 years. The data obtained reveal a mean excretion of acetylated PABA of 0.49 mM (S.E. 0.021) with a range of 0.41–.64 mM (41–64 per cent total drug administered). The individual values are indicated in Figure 1.

In like manner, urine analyses for "free" and "total" excreted PABA were carried out on 17 subjects who had evidence or history of liver disease uncomplicated by renal disease. As with the data from the control subjects, no correlation could be found between either "free" or "total" excreted PABA and urine volume, weight, age or sex. Figure 1 shows, for all subjects, the values of acetylated PABA and the "total" PABA excreted. If the former was a fixed

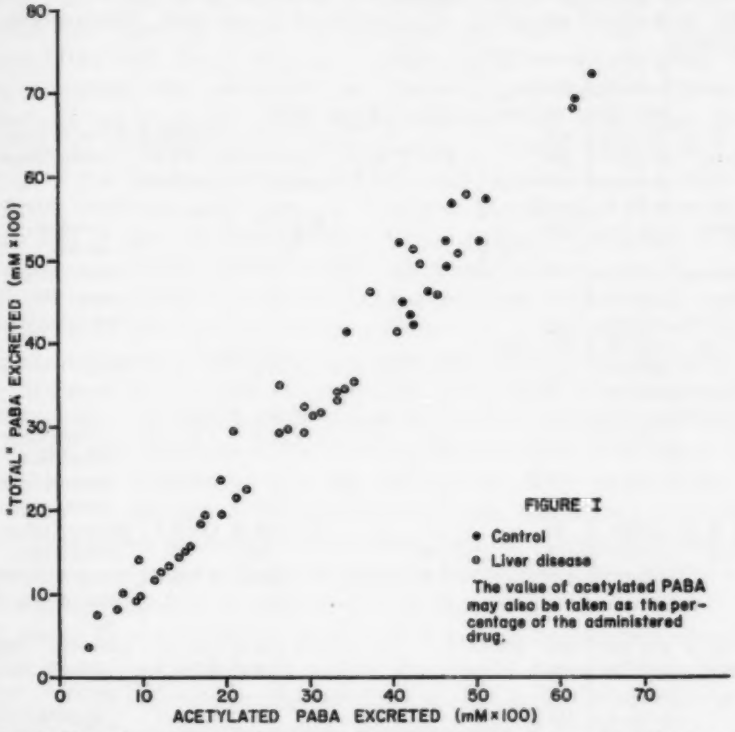

FIG. 1. Excretion of *p*-aminobenzoic acid metabolites by 12 normal subjects and 15 subjects with liver disease during two-hour period after administration of one millimole of *p*-aminobenzoic acid.

percentage of the latter, then the values obtained should describe a straight line from the origin. Examination of the points obtained shown in Figure 1 indicates that in both control and diseased subjects the percentage of the "total" PABA excreted which is acetylated is about 90 ± 10. This comparison, then, shows no striking difference between the two groups. However, further examination of the values shown in Figure 1 shows that the control acetylated PABA points uniformly exceed an absolute value of 0.40 mM or 40 per cent of

TABLE II

Comparative Laboratory Data on Fifteen Patients with Liver Dysfunction

Patient & weight	Date	acetylated PABA in 2 hour urine as per cent PABA adm.	2 hour urine volume	30' BSP	cephalin flocculation	thymol turbidity	serum albumin/globulin ratio	alkaline phosphatase	serum bilirubin total/direct	Diagnosis
			ml.	mg. %		units	grams %	Bodansky units	mg. %	
WEJ 45 cm 138#	3-15	9.7	100	0.5	+	3.4	2.9/2.9	3.6	<0.8	Portal cirrhosis
	3-20	15.1	270							
	6-9	29.5	34	0.7	0	5.9	3.6/3.7			
CY 33 cf	3-14	13.1	410				3.9/3.3	24.2	19.4/13.6	Portal cirrhosis, ? hyperthyroidism
140#	4-10	19.3	615		++	10.5	3.5/3.3	25.5	5.4/4.6	
	6-9	15.6	112	3.8	+	8.8	4.3/1.9	27.6	2.0/1.4	
PM 46 wm	4-24	9.1	175							Portal cirrhosis, tuberculosis, pulmonary
140#	5-1	14.3	140	.6 (45')			4.4/2.6			
JL 49 wm	5-1	34.6	41							Portal cirrhosis, (biopsy) 1948, 1953
220#	5-15	37.4	46	1.5						
CK 55 wm	5-22	31.6	350							Portal cirrhosis, 1954
160#	6-5	40.8	580	1.0						
EH 47 wf	6-5	29.8	565	1.5	+	2.3	4.4/2.2	3.6		Portal cirrhosis (biopsy) 1953
129#	7-2	42.9	50							
MK 52 wf	6-13	7.4	65	5.1	++	2.6	2.4/3.3	11.9	1.6/1.4	Biliary cirrhosis, cholangiolytic with cholelithiasis. Prob. portal cirrhosis
120#	6-16	17.2	40		++	5.4	2.3/4.5	20.1	1.8/1.1	
MG 38 cf	3-22	12.3	90		+++		4.2/6.2	68.4	7.0/5.4	Primary biliary cirrhosis (biopsy)
117#	3-27	11.3	115		+++			69.0		
	5-1	27.7	100		+++	>31.0	2.5/7.9	64.5	8.0/6.0	Transaminase 96 μ/ml. (normal 50 μ/ml.)
EC 45 cm	4-4	26.7	490	4.0	++	7.7	3.9/3.0	13.6	9.2/7.0	Infectious hepatitis
152#	4-11	19.5	640					8.8	9.0/6.2	with ? post-necrotic cirrhosis
	4-18	7.9	90				4.4/2.7		5.9/4.7	
	4-24	4.5	335		+	6.0		7.2	4.0/2.8	? hyperthyroidism
	5-3	9.6	440		0	3.8		6.2	2.8/2.0	
CW 32 cf	3-14	22.6	675		+++	23.0	4.0/3.9	9.8	7.2/5.7	Infectious hepatitis
132#	3-22	43.2	525		+++	19.0			1.4/1.0	
CT 34 cf	4-10	34.5	420		++	3.0	4.1/3.3	16.1	12.5/8.7	Infectious hepatitis
138#	4-18	30.6	310					17.2	14.0/10.2	
	4-24	48.0	235					14.2	7.4/5.4	
	5-3	33.7	355					13.8	4.0/3.5	
SS 29 cf	5-1	3.8	420				3.5/3.1	19.2	14.0/8.0	Homologous serum hepatitis
118#	5-9	45.4	560		++	3.4	4.4/3.2		2.5/1.4	
AG 50 cf	5-9	26.7	235		+++	7.3	4.9/3.2	5.0	7.9/4.0	Homologous serum hepatitis
117#	5-15	21.1	315		++	4.5		6.0	3.5/2.1	
CS 28 cf	5-9	21.3	665		++++	20.0	4.0/5.1	6.5	7.5/3.7	Homologous serum hepatitis
137#	5-16	33.5	590			9.0			4.5/2.3	
LS 67 wf	5-23	17.8	365	1.5	++	1.2	3.2/1.9	15.9	<0.8	Periarteritis nodosa
118#										

the one millimole administered. On the other hand, 33 of the 40 values obtained from the diseased group fall below this 0.40 mM acetylated PABA figure. Thus the two groups appear to be distinct on the basis of the acetylated PABA values alone. This difference becomes more marked when consideration is given to the fact that of the seven points from the "diseased" group exceeding the 40 per cent or 0.41 mM figure, five were obtained from individuals either in the convalescent stage of infectious or homologous serum hepatitis or had recovered from hepatitis within the preceding two years. These five values exceeded 0.43 mM. The remaining two points in this overlapping group were from individuals with portal cirrhosis of several years duration. These two individuals had repeated values ranging from 0.30–0.43 mM. Thus, if one considers the excreted acetylated PABA either in absolute terms or as a percentage of the administered dose of PABA, there appears to be a clear distinction between normal subjects and those with liver disease.

The data obtained from the group with liver disease are presented in Table II, together with such comparative chemical determinations as were available. The cases of portal cirrhosis showed excreted acetylated PABA values varying from 0.09–0.43 mM. The patients bore clinical stigmata of disease in minimal to severe degree which appeared to be rather more accurately reflected by the rate of acetylation than the other chemical data which were obtained. The correspondence between the bromsulfalein (BSP) retention and rate of acetylation, when such comparison was available, was generally good. There were several instances where jaundice was marked and BSP retention was not determined. In the case of WEJ the serum albumin was slightly diminished, the BSP retention was within normal limits, and the acetylated PABA rose gradually over a period of three months from 0.09 mM to 0.30 mM. The rates of acetylation appeared to reflect his clinical picture.

It should be noted that the possibility of hyperthyroidism was suspected in two cases. Since acetylation may be significantly depressed in hyperthyroidism (2) (7), this must be considered as a possibly contributing factor in the depression of the acetylation rates in these two cases, C.Y. and E.C.

The case of periarteritis nodosa (L.S.) showed depression of acetylation rate as well as abnormal values of other tests of liver function.

The cases of infectious and homologous serum hepatitis showed wide variations in rate of acetylation. Unfortunately, there were too few determinations of BSP retention in this group for comparison. Correspondence of acetylation rate with other chemical determinations done was poor. However, most of the patients showed depression of the rate of acetylation in the early phase of disease and a return toward normal rates commensurate with speed of clinical recovery.

DISCUSSION

Bloomberg (1) and Gershberg and Kuhl (2) used a comparison of the acetylated PABA excreted with the "total" PABA excreted, the "total" being the sum of the "free" and acetylated fractions. They found no difference in the per cent excreted which was acetylated in the normal and liver disease groups. Percentagewise, the same was found to be true in this study. The striking point, however, is that both the "free" and "acetylated" absolute amounts excreted were diminished in varying degree in the majority of individuals with hepatocellular damage. The studies of Riggs and Hegsted (4) and Gershberg and Kuhl (2) have shown that the percentage acetylation rises as the administered dose of PABA diminishes. The present study in agreement with the work of Gershberg and Kuhl shows acetylated metabolites to account for about 90–100 per cent of total excreted metabolites. The acetylated metabolites, as indicated by Riggs and Christensen (6), include acetyl PABA and acetyl PAHA. Tabor *et al.* (8), working with considerably larger administered doses of PABA found suggestive evidence that a significant proportion of the PABA excreted was conjugated with glucuronic acid and a high percentage of this conjugate was acetylated. Thus it appears that a very small proportion of PABA administered is excreted unchanged. Conjugation with glycine or glucuronic acid in addition to acetylation occurs. Riggs and Christensen (6) have shown that a reciprocal relationship exists between the degree of acetylation and that of conjugation with glycine. Depression by artificial means of one leads to enhancement of the other. In the absence of artificial suppressive measures, the degree of acetylation is predominant at low doses of PABA and diminishes while the glycine conjugation increases as the dose administered increases. Thus one would suspect that if the rate of acetylation was diminished as the result of disturbance of metabolism in the liver that the conjugation with glycine might be compensatorily increased. However, Tabor *et al.* (3) noted in their brief extract that in their studies in patients with liver dysfunction that there was a marked reduction in PAHA excreted and that in the normal the PAHA fraction of the total "free" metabolites excreted was high. Thus it would appear that conjugation with acetic acid and glycine may be depressed in liver dysfunction. The finding of increased plasma concentration of PABA by Tabor *et al.* (3) would appear to support this thesis. Further definitive differential studies of blood and urine to test this are in progress.

SUMMARY

PABA was administered orally to a group of patients exhibiting a variety of liver diseases. The rate of acetylation of PABA as reflected by the urinary excretion of the acetylated metabolites of PABA over a two-hour period was determined. This rate was found to be lower than control values in the acute phase of infectious and homologous serum hepatitis, portal and biliary cirrhosis,

and in one case of periarteritis nodosa with evidence of liver damage. Where recovery was rapid there was a coincident return to normal values of the acetylation rate. The depression of acetylation in cirrhosis was more persistent. In the cases of portal cirrhosis of many years duration slight to moderate depression was present while other chemical evidence of disease was minimal or lacking, even though clinical stigmata of disease remained.

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THE DEVELOPMENT OF ARTERIAL LESIONS FOLLOWING PROLONGED SENSITIZATION TO BOVINE GAMMA GLOBULIN[1]

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Since the demonstration by Rich and Gregory (1–4) that sensitization of rabbits to foreign serum can lead to the development of a wide variety of allergic lesions, including arteritis, endocarditis and glomerulonephritis, considerable attention has been given to the immunologic and histologic responses to some of the individual serum antigens (5–11). Experiments utilizing purified serum fractions have shown that all of the allergic lesions produced by whole serum can be elicited to a greater or lesser degree by a single serum protein (5–9). However, certain interesting differences have been observed between the immunologic and histologic responses to some of the serum antigens. In an early study on hypersensitivity to purified serum fractions, Hawn and Janeway (5) noted that a single intravenous injection of crystallized bovine albumin produced lesions which were almost wholly confined to the arteries whereas bovine serum gamma globulin induced alterations which were almost exclusively confined to the glomeruli of the kidneys. These workers were able to correlate the histologic differences between these two antigens with a more rapid disappearance of gamma globulin from the circulation and a more rapid evolution of lesions following gamma globulin as compared with albumin.

Recent work (7, 8, 12) has confirmed the findings of Hawn and Janeway indicating that a single injection of bovine gamma globulin leads to the development of glomerular and endocardial lesions but fails to produce extensive arterial alterations. However, studies from this laboratory (9, 12) have not supported their observations concerning a selective histologic effect of bovine albumin for it has been found that marked glomerular lesions as well as vascular alterations can be produced by a single injection of this antigen. The failure of Hawn and Janeway to observe extensive glomerular lesions following albumin might have been due to the fact that their animals were sacrificed in groups at weekly intervals rather than individually at a time when the antigen has just disappeared from the circulation and the glomerular lesions are at their highest incidence (9, 12).

[1] This investigation was supported by a grant (A-715) from the National Institute of Arthritis and Metabolic Diseases, of the National Institutes of Health, Public Health Service.

[2] Eli Lilly Travelling Fellow, Medical Research Council of Great Britain; present address: St. Mary's Hospital, London W.2., England.

At present, the essential difference between the histologic effects of bovine albumin (or whole serum) and bovine gamma globulin is the absence of well marked arterial lesions following a single injection of globulin. Correlated with this difference in histologic effect is a marked difference in the biologic behavior of these two antigens. The catabolic half-life of bovine gamma globulin is 2 days while that of bovine albumin is approximately 4 to 5 days (9, 10, 12). The antibody response to globulin appears considerably earlier than that to albumin (5, 10, 12). Consequently, as Hawn and Janeway originally noted, bovine gamma globulin remains in the circulation on the average for only 1 week after injection instead of approximately 2 weeks, as is the case with bovine albumin.

In a previous study (11), it was found that the severe arterial lesions ordinarily observed in normal rabbits following a single injection of albumin, were infrequently encountered in previously sensitized rabbits where the rate of removal of albumin from the circulation was markedly accelerated by the anamnestic response. This observation suggested the possibility that the failure of a single injection of bovine gamma globulin to produce well marked arterial lesions in normal rabbits might be related to the short interval that this antigen remains in the circulation as a result of its greater catabolic rate.

The present study was undertaken to determine whether increasing the length of exposure to bovine gamma globulin would result in the production of arterial lesions. For this purpose, rabbits were given single and multiple injections of bovine gamma globulin.

MATERIALS AND METHODS

Male albino rabbits of approximately 2 kg. weight were used throughout the experiment. Because of the report that the removal of one kidney increased the severity of glomerular lesions following bovine gamma globulin (7), one kidney was removed in half of the animals.

Group I consisted of 10 rabbits, half of which had one kidney surgically removed between 9 and 21 days previously. These animals received a single intravenous injection of 2 gm. of bovine serum gamma globulin (Armour & Co.) dissolved in 20 ml. of isotonic saline with sufficient sodium bicarbonate added to adjust the pH to 7.4. This dose of gamma globulin has been found to give the highest incidence of lesions (12). Daily tests for the presence of circulating bovine gamma globulin were made with potent antiserum using the capillary tube method as described in detail elsewhere (12). Rabbits were killed by air embolism as soon as the circulating antigen had disappeared. At this time, the lesions are known to be of highest incidence and severity (9).

Group II consisted of 16 rabbits, half of them with one kidney removed as before. These rabbits were given an initial injection of bovine gamma globulin as in Group I, and this treatment was repeated whenever the circulating antigen as shown by the capillary tube test became negative. In this way, the rabbits received additional antigen at a rate according to their ability to eliminate the preceding dose. The antigen was given in this manner rather than at fixed intervals in order to avoid immune blackout due to excessive accumulation of antigen or anaphylactic shock due to formation of high levels of circulating antibody between injections. This treatment was carried out for a period of 5 weeks during which time

each animal received between 3 and 7 injections. Two animals, both with one kidney removed, which died early in the experiment are omitted from the data. Three died during the last few days of the experiment, and the remainder were killed by air embolism on the 34th day.

For controls, 8 rabbits were injected with 20 ml. of isotonic saline at weekly intervals. Four of these had had one kidney removed. These animals were killed on the same day as the experimental animals.

Because glomerular lesions were anticipated, daily blood pressure estimations were made on animals for several days prior to the start of the experiment and during the entire course of the experiment. These determinations were made on the central artery of the ear, using a Grant-Rothschild capsule (13), and observing the precautions advocated by Pickering and Prinzmental (14). Urine was tested for protein each day by heating, after the addition of acetic acid. The rabbits were fed on a pelleted diet with unlimited water, and were weighed twice weekly. Postmortem examinations were carried out on all animals. The heart,

TABLE I

Histologic Changes in Rabbits Receiving a Single Injection of Bovine Gamma Globulin

Rabbit No.	Day Sacrificed*	Endocarditis	Arteritis		Glomerulonephritis
			Intimal	Necrotizing	
With both kidneys					
368	8	0	0	0	+
380	8	0	+ aorta	0	+
388	8	0	0	0	0
367	13	0	0	0	0
370	19	0	0	0	0
With one kidney					
366	9	+	0	0	0
377	9	0	0	0	+
389	9	0	0	0	+
365	11	+	0	0	0
436	11	0	+ aorta	0	0

* Each animal was sacrificed when test for serum antigen became negative.

adrenals, spleen and kidneys were weighed. Routine histologic preparations were prepared from the heart and root of aorta, lungs, liver, spleen, adrenals, pancreas, kidneys, mesenteric lymph nodes, mesentery and intestine.

EXPERIMENTAL RESULTS

Pathologic Findings

A summary of the pathologic findings is given in Tables I and II. As there were neither qualitative nor quantitative differences between rabbits with one and those with two kidneys, the pathology of the two is discussed together.

Group I. Allergic lesions were not prominent in animals receiving a single injection of bovine gamma globulin. The most frequent alteration consisted of a proliferative glomerulonephritis in four of the ten animals, characterized by a slight to moderate increase in the number of cells of the gomerular tufts. No

necrotizing or inflammatory lesions of the medium sized arteries were encountered. However, two rabbits showed moderate collections of mononuclear cells beneath the endothelium of the root of the aorta and two others showed minimal inflammatory changes in the heart valves.

Group II. In contrast to the paucity of lesions in rabbits receiving a single intravenous injection of bovine gamma globulin, animals given multiple injections showed striking cardiovascular alterations.

TABLE II

Histologic Changes in Rabbits Receiving Multiple Injections of Bovine Gamma Globulin

Rabbit No.	No. of Injections	Day Sacrificed	Endocarditis	Arteritis		Glomerulonephritis
				Intimal or Adventitial	Necrotizing	
With both kidneys						
70	7	30*	+	+ lung, heart, mesentery, liver	+ lung, heart, mesentery, liver	0
82	5	33*	+	+ aorta, lung, heart	0	+++
69	5	34	+	+ aorta	0	+
75	4	34	+	0	0	0
83	4	34	+++	+ heart, mesentery	+ heart	0
85	4	34	+++	+ heart	0	0
78	3	30*	+	0	0	0
87	3	34	+	+ heart	+ heart	+
With one kidney						
93	5	34	+++	+ heart, liver	0	0
95	5	34	+++	+ lung, heart	+ heart	0
97	5	34	++	+ heart	0	+
99	5	34	+	0	0	+
129	5	34	++	+ heart,	0	0
131	4	34	+++	+ heart	0	0

* Died

Endocarditis and glomerulonephritis graded 0 to +++; otherwise + = presence of lesion.

An endocarditis involving either the mitral or aortic valve or both was present in all animals (Figs. 1, 2). This lesion consisted of marked proliferation of the endothelial cells and subendothelial infiltration by mononuclear cells, some of which were multinucleated. Occasionally, large numbers of lymphocytes were present. The entire substance of the most severely affected valves appeared to be involved in the inflammatory reaction. The valve insertions were often involved in a similar process. In no instance were amorphous vegetations or fibrinoid alterations encountered.

Arterial lesions were present in 11 out of 14 rabbits (Figs. 3, 4). In 7 of the 11 rabbits with arterial lesions, the inflammatory changes were confined to the intima or adventitia, although rare mononuclear cells were sometimes seen

in the media. In the remaining 4 rabbits with arterial lesions, there was obvious necrosis of the vessel wall with marked mononulcear cellular infiltration. The coronary arteries were most commonly affected. An unanticipated finding was the absence of a high incidence of well marked glomerular lesions. A pronounced glomerulonephritis was seen in only one rabbit (Fig. 5). This lesion consisted of marked proliferation of glomerular nuclei and focal necrosis of the tufts. In 4 other animals, there were only minimal changes consisting of increase in nuclei and slight capsular adhesions. In addition to the above mentioned

TABLE III

Immunologic Response with Multiple Injections of Bovine Gamma Globulin Given Over a Period of 35 Days

Rabbit No.	Total No. of Injections	Intervals in days between injections						Last Inj. & Death
		1 & 2 inj.	2 & 3	3 & 4	4 & 5	5 & 6	6 & 7	
With both kidneys								
70	7	8	5	4	3	3	6	1*
82	5	8	5	6	14			0**
69	5	9	4	5	5			11
75	4	9	8	11				6
83	4	8	6	9				11
85	4	10	8	8				8
78	3	10	11					9
87	3	10	11					13
With one kidney								
93	5	10	6	6	6			6
95	5	9	6	6	6			7
97	5	10	7	7	9			1
99	5	9	7	7	8			3
129	5	9	5	5	6			9
131	4	9	10	12				3

* Delayed death following anaphylactic shock.
** Immediate death following anaphylactic shock.

lesions, one animal showed epithelioid cells in the mesenteric lymph nodes, similar to that described following albumin (12). The lungs of two animals (#82 and #70) which had died of anaphylactic shock following the 5th and 7th injections of bovine gamma globulin respectively showed numerous hyaline and amorphous thrombi plugging dilated alveolar capillaries (Fig. 6). Studies on the origin and role of these thrombi in anaphylactic shock in the rabbit are in progress. No remarkable abnormalities were seen in any of the control animals.

Urinary and Blood Pressure Findings:

All experimental animals except one showed trace amounts of proteinuria at some stage of the experiment. In only 4 was the proteinuria greater than a

trace. In 2 of these animals (#70 and #78) there was spontaneous disease of the kidneys. Another (#69) showed minimal glomerular changes and another (#82) showed marked glomerulonephritis.

No rise in blood pressure was noted in any of the animals except in the case of number 82 which showed a rise of 15 mm. Hg above pre-treatment levels. This rise commenced after 16 days of treatment during which time 3 injections of bovine gamma globulin had been given.

Immunologic Findings

Table I shows the day on which the rabbits of Group I were killed, i.e., the time at which circulating antigen had disappeared following a single intravenous injection. In the majority of animals, the antigen was eliminated in 8 or 9 days, indicative of an adequate immune response (12).

Table III shows the number of injections of antigen given to each animal in Group II, and the interval between each injection. The time required to clear the antigen usually became shorter after the first injection of antigen when antibody production had been established. However, after several injections, the number varying with each animal, there was a tendency for less rapid elimination of antigen as was found to be the case with repeated injections of albumin (15).

DISCUSSION

The present data on the histologic effect of a single injection of bovine gamma globulin is in agreement with results previously obtained in this laboratory (12). A slight glomerulonephritis was the most frequent alteration encountered; endocardial lesions were rare; and arterial lesions were absent.

In contrast to the low incidence of endocardial lesions and absence of vascular lesions in animals given a single injection of globulin, rabbits receiving multiple injections of this antigen over a 34 day period showed marked arterial and valvular alterations. The arterial lesions were comparable in intensity and incidence to those observed in animals similarly treated with albumin (16) while the valvular lesions appeared to be more severe.

Although prolonged sensitization led to marked cardiovascular alterations, changes in the glomeruli of the kidneys were no more striking than after a single injection. Since glomerular lesions were the most prominent alterations produced by a single injection of globulin (present data and 5, 7, 12), the failure of multiple injections of globulin to elicit more extensive glomerular abnormalities was unexpected. However, a more recent study in this laboratory (16) has not only confirmed this finding but in addition has shown that the high incidence of glomerulonephritis ordinarily produced by a single injection of bovine albumin is no longer observed after multiple injections extending over a 35 day period.

The reason for the failure of repeated large injections of antigen to produce progressive glomerular injury while giving rise to severe arterial and valvular damage is not clear. It is of interest that by employing a different method of antigen administration, consisting of small daily injections of horse serum given over many months, McLean and his colleagues (17) were able to produce progressive glomerular disease without the occurrence of arteritis. These

TABLE IV

Effect of Length of Sensitization on Occurrence of Allergic Lesions

	Duration of Circulating Antigen, days	Day of Antibody Formation*	Length of Antigen-Antibody Interaction, days	Arteritis	Glomerulonephritis‡
Single Injection					
BA (sensitized)**.....	6	0	6	−	+
BGG (normal).....	10†	4	6	−	+
BA (normal).....	15†	6	9	+	+
Multiple Injections					
BGG.....	34	4	30	+	−
BA.....	35	6	29	+	−

* Day of antibody formation refers to the time when circulating antigen begins to disappear suddenly due to the immune response. Length of antigen-antibody interaction is the period from the time when circulating antigen begins to disappear more rapidly as a result of the immune response to the time when circulating antigen disappears completely.

** Data from following: Single injection: BA (sensitized) (11)
BGG (normal) (present data and 12)
BA (normal) (12)
Multiple injections: BGG (present data and 16)
BA (16)

† Longest length of time antigen can exist in the circulation when immobilized by an immune response adequate to produce allergic lesions (12).

‡ + = lesion present; − = lesion absent or inconspicuous in comparison to others.

different results suggest that the method of antigen administration and antigen dosage may influence the type of allergic lesion which develops during prolonged sensitization. This matter is presently under investigation.

The effect of the length of sensitization to albumin and globulin on the distribution of allergic lesions is shown in Table IV, where the results of the present study are summarized together with information obtained in this laboratory on the immunologic and histologic responses to single and multiple injections of bovine albumin in normal rabbits (12, 16) and to a single injection of bovine albumin in previously sensitized animals (11). Following a single injection of 2 gms. of bovine gamma globulin (BGG)[3], the longest length of time during

[3] These dosages of globulin and albumin produce the highest incidence of allergic lesions (12).

which the antigen can be detected in the circulation in the presence of an immune response sufficient to produce allergic lesions has been found to be 10 days (12). For 0.5 gm. of bovine albumin (BA)[1], this period has been found to be 15 days (12). The different lengths of time during which these two antigens exist in the body arise from two factors: first and more important, as a result of a faster catabolism, the amount of globulin in the body is reduced by one-half every 2 days instead of every 4–5 days as is the case with albumin (10, 12); secondly, the immune response to globulin is earlier and more rapid (5, 10, 12). Since globulin remains in the circulation for a shorter length of time, the period of antigen-antibody combination following a single injection of globulin is not as long as that following a single injection of albumin (6 and 9 days, respectively).

The possibility arose that the failure of a single injection of globulin to produce marked arterial lesions might be due to the short time that this antigen remains in the circulation. The data collected in Table IV is in keeping with this possibility. When the length of action of a single injection of albumin is shortened such as occurs in previously sensitized animals where the antigen is rapidly immobilized by the anamnestic response, severe arterial lesions are almost as infrequent as in normal rabbits receiving a single injection of globulin (11); on the other hand, when serum levels of globulin are prolonged by repeated injections of this antigen, arterial lesions are as prominent as in animals similarly treated with albumin (present data and 16). These results indicate that the distribution of allergic lesions may be markedly influenced by the length of antigenic exposure and suggest that the differences in distribution of allergic lesions observed following single injections of albumin and globulin may be due to the different lengths of time that these antigens remain in the circulation rather than to fundamental differences in the tissue affinity of these two antigens.

SUMMARY

1. Rabbits were given repeated intravenous injections of bovine gamma globulin at a dosage of approximately 1 gm. per kg. extending over 35 days and the allergic lesions obtained were compared with those produced following a single injection.

2. Following a single injection of bovine gamma globulin, a mild glomerulonephritis developed almost exclusively and arterial lesions were absent. Following multiple injections, prominent valvular and arterial alterations appeared while glomerulonephritis was less frequent. The distribution of allergic lesions following multiple injections of globulin is similar to that following multiple injections of albumin given for the same length of time.

3. A comparison of the present results with data from other experiments suggests that the differences in the distribution of lesions following a single

injection of bovine gamma globulin on the one hand and bovine albumin on the other are due largely to differences in the duration of these antigens in the body as a result of their different catabolic rates and different immune responses. These histologic differences tend to disappear when blood levels of each antigen are maintained for similar lengths of time.

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EXPLANATION OF ILLUSTRATIONS

All photographs are of lesions encountered in animals receiving multiple injections of bovine gamma globulin.

FIG. 1. Rabbit 83 sacrificed after 4 injections of bovine gamma globulin in 34 days. Section of mitral valve and valve ring showing endothelial proliferation, subendothelial mononuclear infiltration and mononuclear cellular infiltration of stroma. Hematoxylin and eosin. × 100.

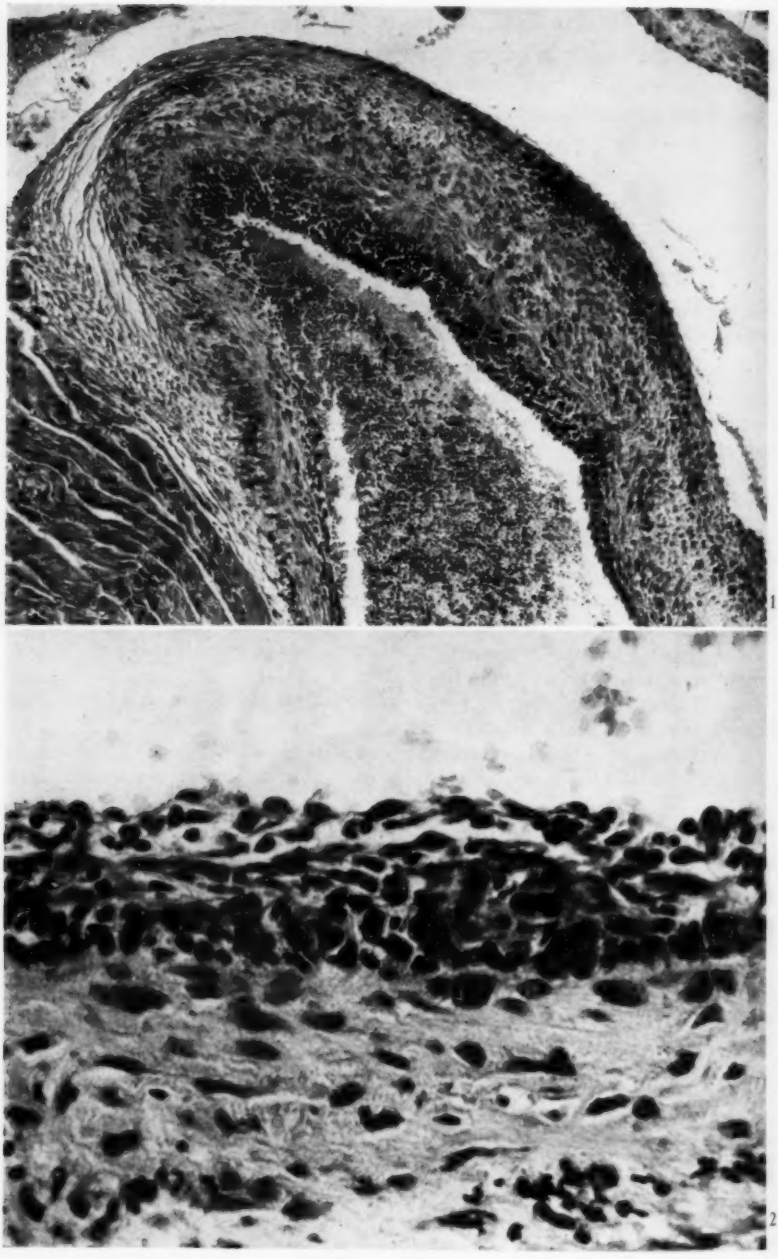
FIG. 2. Rabbit 85, sacrificed after receiving 4 injections of bovine gamma globulin in 34 days. Section of mitral valve showing proliferation of endothelium and subendothelial infiltration by mononuclear cells, some of which are multinucleated. Hematoxylin and eosin. × 500.

FIG. 3. Rabbit 70, died in anaphylactic shock after the 7th injection of bovine gamma globulin. 30 days. Section of coronary artery showing necrosis and disorganization of wall and adventitial and perivascular cellular infiltration. Hematoxylin and eosin. × 250.

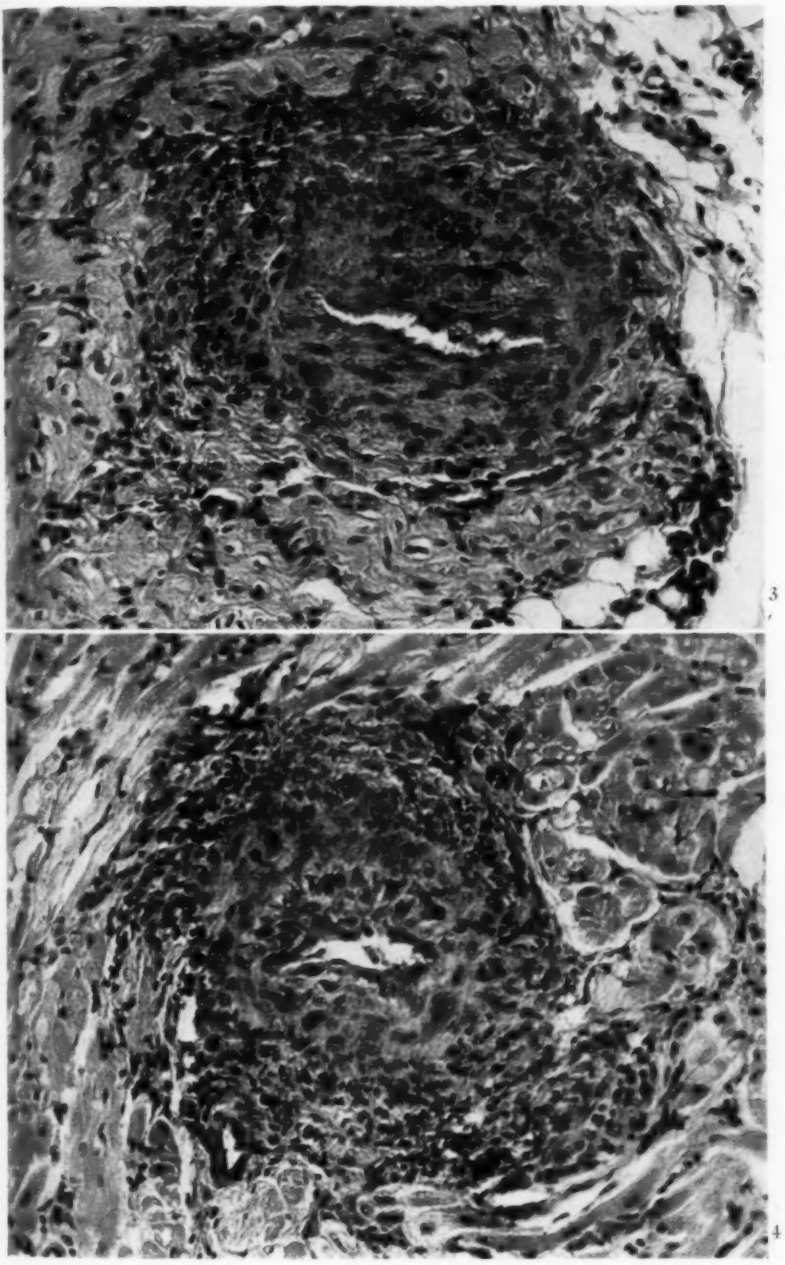
FIG. 4. Rabbit 95, died after 5 injections of bovine gamma globulin in 34 days. Section of coronary artery. Hematoxylin and eosin. × 250.

FIG. 5. Rabbit 82, died after 5 injections of bovine gamma globulin in 33 days. Diffuse glomerulonephritis and casts in tubules. Hematoxylin and eosin. × 150.

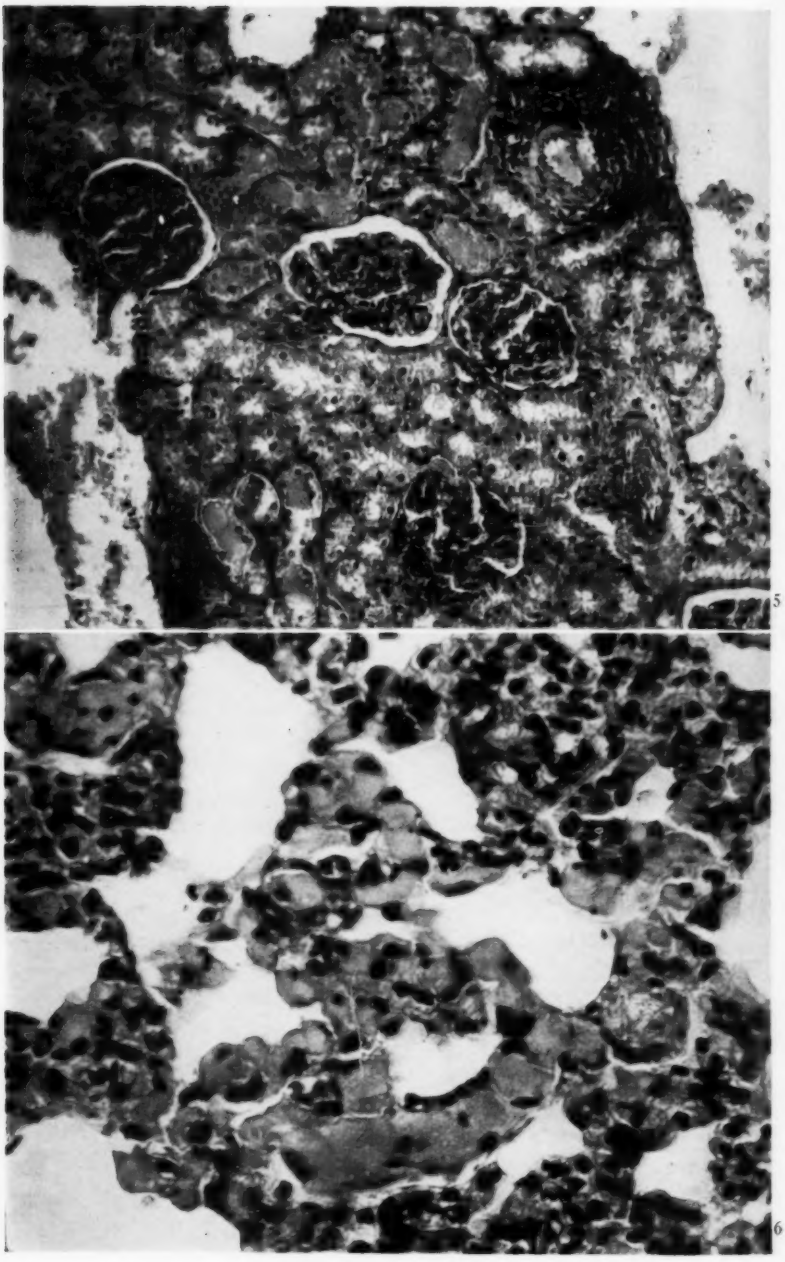
FIG. 6. Rabbit 70, died in anaphylactic shock after the 7th injection of bovine gamma globulin. Dilated alveolar capillaries plugged with hyaline and amorphous thrombi. Hematoxylin and eosin. × 500.



FIGS. 1 AND 2



FIGS. 3 AND 4



FIGS. 5 AND 6

EXPERIMENTAL STUDIES ON THE IMMUNOLOGIC AND HISTOLOGIC EFFECTS OF PROLONGED EXPOSURE TO ANTIGEN

I. Distrubution of Allergic Lesions Following Multiple Injections of Bovine Albumin, Bovine Gamma Globulin, and Albumin and Globulin Together with Special Reference to the Occurrence of Granulomatous Arteritis[1]

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This study is one of a series designed to determine the histologic and immunologic effects of prolonged antigenic exposure. The present experiment deals with the immunologic response and distribution of allergic lesions following multiple injections of bovine albumin, bovine gamma globulin and albumin and globulin together, given over a period of 35 days.

The histologic and immunologic sequences produced by one or two injections of foreign protein have been extensively studied (1–4). The marked similarities between the lesions produced by sensitization to foreign proteins and those of human periarteritis nodosa and glomerulonephritis have been clearly demonstrated by Rich and his colleagues (5, 6), and others (2, 4, 7–10); and the role of hypersensitivity in some cases of periarteritis nodosa has been well established. Certain differences can be noted between the experimentally induced lesions and their human counterparts. Some of these differences may be attributed to species; others may be due to the fact that the allergic reaction produced by one or two injections of foreign protein is so evanescent that the progressive anatomic alterations characteristic of fatal periarteritis nodosa and glomerulonephritis in man are not often encountered in the experimental animal.

To what extent the length of sensitization may modify the character and distribution of allergic lesions is not fully understood. Several studies have been undertaken to determine the histologic effects of prolonged sensitization. Hamilton-Paterson and Henderson (10) observed a high incidence of acute and chronic glomerular lesions and fresh and healed coronary arterial lesions in rabbits given weekly injections of large amounts of horse serum for periods up to 7 months. However, Orbison (11) was unable to elicit progressive allergic

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lesions in rabbits by the administration of large doses of horse serum at semimonthly and monthly intervals for periods of 2 to 6 months. By using a different method of antigen administration, consisting of small daily injections of horse serum for periods ranging from 3 to 13 months, McLean and his colleagues (12) produced extensive glomerulonephritis in the absence of arterial lesions. The widely different results obtained in these studies have emphasized the profound influence that the antigen dosage and the schedule of antigen administration may have on the character and distribution of allergic lesions.

One of the problems encountered in planning the present experiment was the selection of a suitable schedule of antigen administration. When injections of foreign protein are spaced far apart, there may be long periods during which insufficient antigen is present to insure continued sensitization. On the other hand, frequent large injections of foreign protein may produce such high levels of circulating antigen that antibody formation is inhibited and serum sickness fails to occur (13). The development in this laboratory of a simple method for the detection of circulating antigen provided a technique by which the course of antigen in the blood could be readily followed in large numbers of rabbits (14). Accordingly, it was possible to employ a schedule of antigen administration which consisted of the reinjection of foreign protein as soon as the antigen from the preceding injection had disappeared from the blood. By this method, more or less continuous sensitization was maintained.

MATERIALS AND METHODS

In a pilot study, 16 male albino rabbits weighing 2 to 2.5 kg. were injected intravenously with 0.5 gm. of amorphous bovine serum albumin (Pentex, Inc.)[3] dissolved in 5 ml. of physiological saline and with 2.0 gm. of bovine gamma globulin (Armour & Co.) dissolved in 20 ml. saline. The solutions had been adjusted to pH 7.4 with solid sodium bicarbonate. Two animals died within 1 day following the injections. The blood of the remaining 14 animals was tested daily for the presence of both bovine albumin and bovine gamma globulin by the use of potent antisera to *crystallized* bovine albumin (Armour and Co.) and antisera to bovine gamma globulin. These tests were made by the capillary tube method as described elsewhere (14). Whenever the test for either circulating antigen was negative, a further injection of that particular antigen was administered at the same dosage as before. This procedure was carried out for 48 days, by which time only 2 animals were still alive, the remainder having died chiefly from anaphylactic shock.

For the main experiment, 25 rabbits were given intravenous injections of amorphous bovine serum albumin and bovine gamma globulin as before. During the early days of the experiment, following successive injections of antigen, numerous rabbits died from anaphylactic shock.[4] In order to avoid this difficulty, the method of administration of antigen was changed after the 25th experimental day. Instead of the intravenous injection of the entire amount of antigen, one-fifth of the dosage (0.1 gm. of bovine albumin or 0.4 gm. of bovine gamma globulin) was given intraperitoneally and the remainder administered intravenously 2 hours later. This procedure seemed to prevent death from anaphylaxis.

[3] Purity greater than 95 per cent by electrophoretic analysis.

[4] Histologic changes related to shock will be described elsewhere.

TABLE I

Histologic Alterations in Rabbits Receiving Multiple Injections of Bovine Albumin

TABLE I

Histologic Alterations in Rabbits Receiving Multiple Injections of Bovine Albumin

Rabbit No.	Albumin, No. of Injections	Day, Dead or Sacrificed	Arteritis					Endocarditis	Glomerulonephritis	Granulomatous Lesions	
			Subendothelial Infiltration	Acute Necrotizing	Partially Healed Necrotizing	Healing*	Healed			Spleen	Lymph Nodes
948	2	10 D†	+ lung, aorta	0	0	0	0	0	+	+	0
931	4	35	0	0	0	0	0	0	0	0	0
961	7	35	+ lung, aorta	heart, mesentery, + kidney: hilar, *arcuate, interlobular*	heart, mesentery, + kidney: hilar, *arcuate, interlobular*	0	0	0	+	0	+
916	9	35	+ lung	0	0	+ heart mesentery	+ heart mesentery	0	0	0	0
920	10	35	+ lung, aorta	0	0	heart + stomach kidney, hilar	0	+	0	0	+
949	10	35	+ lung	0	+ kidney: hilar, *arcuate*	+ heart mesentery	+ heart mesentery	0	0	0	0
913	10	35	0	0	0	0	+ mesentery	0	0	0	0
942	12	35	+ lung, aorta	0	0	+ heart	+ heart	0	0	0	0
923	13	35	+ lung, aorta	0	0	0	0	+	0	–	0
937	13	35	+ lung, aorta	+ kidney, hilar	+ kidney, hilar	+ heart mesentery	+ heart mesentery	0	+	0	0
Total = 9‡			7(78%)	2(22%)	3(33%)	5(56%)	5(56%)	2(22%)	2(22%)	0	2(22%)

* Differs from partially healed necrotizing arteritis by the absence of discernible necrosis.

† D = died.

‡ Rabbit 948 dying early in the experiment not included.

In addition to the 25 rabbits receiving both antigens, 10 rabbits received repeated injections of 0.5 gm. of amorphous bovine albumin only, and 12 others received repeated injections of 2 gm. of bovine gamma globulin only. Animals not dying of shock were killed by air embolism at the end of 5 weeks.

Complete autopsies were performed and histologic preparations were made of all important organs.

EXPERIMENTAL RESULTS

The histologic findings are recorded in Tables I–IV and summarized in Table V. In Tables I–IV, the animal numbers are arranged according to the length of survival. In Table I are recorded the histologic findings in animals receiving multiple injections of bovine albumin, and in Table II the findings in animals receiving multiple injections of bovine gamma globulin. Animals receiving both antigens have been divided into two groups for analysis: Table III comprises animals surviving up to 19 days; Table IV contains those surviving for longer periods. The former group showed only acute arterial lesions, while the latter also showed healing and healed arteritis. In all tables, the number of injections of bovine albumin or gamma globulin or both is recorded for each animal.

A. Pathologic Findings

In the experimental animals, lesions were encountered in the aorta, in the medium sized and small arteries (Figs. 1, 2, 5–15), in the cardiac valves, in the glomeruli of the kidneys (Fig. 3), and in the lymphoid tissue of the spleen (Fig. 4) and lymph nodes. Similar alterations were not observed in 10 control rabbits.

1. Glomerulonephritis. The most striking glomerular alterations were observed in the group of 17 animals receiving both bovine albumin and bovine gamma globulin and dying within the first 19 days of the experiment. Nine or 53 per cent of these animals showed definite glomerular lesions (Table III). All of the glomerular lesions were acute in nature and consisted of varying degrees of leucocytic infiltration and proliferation of the cellular elements of the capillary tufts. In addition, 2 animals showed extensive focal necrotizing as well as proliferative lesions (Fig. 3).

In contrast to the high incidence of glomerulonephritis in this group, animals surviving for longer periods of time showed very few glomerular alterations despite the continuous injection of albumin, globulin or both these antigens. Only 2 of 9 (22 per cent) bovine albumin-treated animals surviving for 35 days showed glomerular changes; only 2 of 10 (20 per cent) bovine gamma globulin-treated animals surviving for 34–35 days showed lesions; and glomerular lesions were scarce in the animals treated with both antigens and surviving for 22 to 48 days (Tables I, II, and IV). No progressive glomerulonephritis was encountered, although occasional capsular adhesions and subcapsular

TABLE II

Histologic Alterations in Rabbits Receiving Multiple Injections of Bovine Gamma Globulin

Rabbit No.	Globulin, No. of Injections	Day, Dead or Sacrificed	Arteritis					Endocarditis	Glomerulonephritis	Granulomatous Lesions	
			Subendothelial Infiltration	Acute Necrotizing	Partially Healed Necrotizing	Healing	Healed			Spleen	Lymph Nodes
944	2	9 D	0	0	0	0	0	0	0	0	0
951	3	18 D	0	0	0	0	0	+	0	0	0
955	5	34	0	0	0	0	0	0	0	0	+
925	5	34	0	0	0	0	0	+	0	0	0
924	5	34	lung, + heart, aorta	0	0	0	0	+	0	0	0
914	6	34	+ lung, aorta	0	heart, + stomach, mesentery	0	0	+	+	0	0
918	6	34	+ lung	0	0	+ heart	0	+	0	0	0
929	6	34	+ lung, aorta	0	+ heart	0	0	+	0	0	0
935	6	34	+ aorta	0	0	+ heart	+ heart	+	+	0	0
962	6	34	+ aorta	0	heart, + lung, kidney, hilar	0	0	0	0	0	0
963	6	35	+ aorta	0	0	+ heart	+ heart	+	0	0	0
921	7	34	+ lung, aorta	0	0	heart, + kidney, hilar	+ heart	+	0	0	0
Total = 10*			8(80%)	0	3(30%)	4(40%)	3(30%)	8(80%)	2(20%)	0	1(10%)

* Does not include 944 and 951 which died early in the experiment.

TABLE III

Histologic Alterations in Rabbits Dying Within 19 Days After Receiving Both Bovine Albumin and Bovine Gamma Globulin

Rabbit No.	No. of Injections		Day, Dead or Sacrificed	Arteritis					Endocarditis	Glomerulonephritis	Granulomatous Lesions	
	Albumin	Globulin		Subendothelial Infiltration	Acute Necrotizing	Partially Healed Necrotizing	Healing	Healed			Spleen	Lymph Nodes
353†	1	2*	7D	+ lung	0	0	0	0	0	+++	++	0
922	2*	1	10D	+ lung, aorta	+ mesentery	0	0	0	+	+++	0	0
936	2*	1	10D	+ lung, aorta	+ heart, mesentery, kidney, hilar	0	0	0	+	+++ nec.‡	+	+
945	2	2*	10D	+ lung, aorta	0	0	0	0	+	0	0	0
947	1	2*	10D	+ lung, aorta	0	0	0	0	+	+++	+	0
952	2	2*	10D	+ lung, aorta	+ heart, stomach	0	0	0	+	+	+++	0
953	2*	1	10D	+ lung, aorta kidney, hilum	0	0	0	0	0	+++ nec.	+	0
979	2*	1	10D	0	0	0	0	0	0	0	0	0
930	2	2*	11D	+ lung, aorta	0	0	0	0	+	+	0	0
950	2*	2	11D	+ lung, aorta	0	0	0	0	0	0	0	0
355†	2	3*	12D	+ lung	+ heart, mesentery, kidney, hilar	0	0	0	+	0	+	+
943	2*	2*	12D	+ lung, aorta	0	0	0	0	0	0	0	0
964	2*	2*	13D	+ heart	+ mesentery	0	0	0	+	++	0	0

943	2*	2*	12D	+ lung, aorta	0	0	0	0	0	0	0	0
964	2*	2*	13D	+ heart	+ mesentery	0	0	0	+	++	0	0
350†	2	3*	14D	+ aorta	+ heart, liver	0	0	0	0	++	+	0
927	2*	2	15D	+ lung	0	0	0	0	0	0	0	0
342†	2	3	17D	0	0							
344†	2	3	19D	+ lung	0	+ heart	0	0	+	0	0	0
Total = 17				15(88%)	6(35%)	1(6%)	0	0	9(53%)	9(53%)	7(41%)	2(12%)

* Died within 24 hours following injection of one or both antigens; many exhibited shock and died immediately after injection.

† Animals in pilot experiment.

‡ Glomerulonephritis and splenic lesion graded 1+ to 3+. Nec. = Focal necrotizing in addition to proliferative glomerulonephritis.

TABLE IV

Histologic Alterations in Rabbits Surviving 22 to 48 Days with Multiple Injections of Both Bovine Albumin and Bovine Gamma Globulin

Rabbit No.	No. of Injections		Day Dead or Sacrificed	Arteritis					Endocarditis	Glomerulonephritis	Granulomatous Lesions	
	Albumin	Globulin		Subendothelial Infiltration	Acute Necrotizing	Partially Healed Necrotizing	Healing†	Healed			Spleen	Lymph Nodes
938	3	3*	22D	+ lung aorta	+ heart	0	0	0	+	0	0	0
946	3	4*	22D	+ aorta	+ lung, heart, stomach, intestine, mesentery, kidney: hilar, *arcuate*	+ lung, heart, stomach, intestine, mesentery, kidney: hilar, *arcuate*	0	0	+	0	0	0
966	4*	4*	23D	0	0	0	0	+ heart	0	0	0	0
915	5	5*	24D	+ lung	+ heart mesentery kidney: hilar	+ heart mesentery	0	0	+	0	0	+
354**	2	4*	30D	+ aorta	+ kidney: hilar, *arcuate, interlobular*	+ heart, intestine, mesentery, kidney: hilar, *arcuate, interlobular*	0	+ mesentery	+	0	+	0
356**	3	5*	30D	0	+ heart, kidney: hilar, *arcuate, interlobular*	+ heart, mesentery, intestine, kidney: *arcuate, interlobular*	0	+ heart, mesentery	+	0	0	0
347**	2	4*	31D	0	0	0	0	0	0	0	0	0
919	14	5	32	+ heart	0	0	+ heart	0	+	0	0	0
345**	6*	5*	33D	0	+ mesentery	0	+ heart	+ heart	+	+	0	0

345**	6*	5*	33D	0	+ mesentery	0	+ heart	+ heart	+	+	0	0
348**	3	4*	33D	+ lung	+ mesentery	heart, + kidney: hilar	0	0	+	0	0	0
912	6	4	35	+ lung aorta	0	mesentery + kidney: *interlobular*	+ heart liver	heart, + mesentery, liver	+	0	0	0
926	5	3	35	+ aorta	0	0	0	+ heart	+	0	0	0
928	3	3	35	0	0	0	0	0	0	0	0	0
932	6	4	35	+ lung aorta	0	+ heart	stomach, mesentery, + bladder, kidney: *interlobular*	heart, liver, + mesentery, bladder	+	+	0	0
941	8	4	35	+ lung aorta	0	0	+ heart	+ heart	+	+	0	0
965	8	5	35	0	0	0	0	+ mesentery	0	0	0	+
967	3	4	36	0	0	0	0	0	0	0	0	0
340**	4	4	42D	0	0	0	+ heart, liver, mesentery	heart, liver, + mesentery, kidney: hilar	+	0	0	0
351**	5	4*	44D	0	+ heart	+ heart mesentery	0	+ heart mesentery	+	+	+	+
343**	5	4	48	0	0	0	0	+ heart	0	0	0	0
346**	5	6	48	0	+ mesentery	+ heart liver	+ kidney: *interlobular*	+ heart mesentery	0	0	0	0
Total = 21				10(48%)	9(43%)	9(43%)	7(33%)	13(62%)	14(67%)	4(19%)	2(10%)	3(14%)

* Died within 24 hours following injection of one or both antigens; most of these animals exhibited shock following antigen injection.

** Animals in pilot experiment.

† Differs from partially healed necrotizing arteritis by the absence of discernible necrosis.

‡D = Died; others sacrificed.

crescents were observed in otherwise normal kidneys. The data for the group is summarized in Table V.

2. Splenic and Lymph Node Lesions. Granulomatous lesions of the Malpighian bodies of the spleen (Fig. 4) identical to those described in preceding studies (4, 15) were frequently encountered in the group of animals dying

TABLE V

Summary of Incidence of Allergic Lesions

Antigen	Time of Death or Sacrifice	No. of Rabbits	Per cent of Animals with			Splenic Lesion
			Arteritis	Endocarditis	Glomerulonephritis	
Both bovine albumin and gamma globulin	7–19 days; Av. = 12 days	17	41	33	53	41
	22–48 days; Av. = 34 days	21	86	67	19	10
Bovine albumin only	35 days	9	78	22	22	0
Bovine gamma globulin only	34–35 days	10	70	80	20	0

early in the experiment following multiple injections of both bovine albumin and bovine gamma globulin (Table II and summary in Table V). As with the glomerular lesions, the splenic lesions were infrequent in animals dying after 3 weeks, regardless of the type of antigen administered. Granulomatous lesions of the lymph nodes were never prominent in any of the experimental groups.

3. Endocarditis. Changes in the valve cusps and in the valve insertions took the form of proliferation of endothelial cells and subendocardial infiltration with large mononuclear cells and lymphocytes. These changes have been described in detail elsewhere (16). The valvular lesions tended to increase in incidence with increasing numbers of injections. Thirty-three per cent of the animals receiving both albumin and globulin and dying early in the experiment showed valvular lesions; however, of those surviving beyond this period, 86 per cent developed such lesions (Table V). Animals receiving multiple injections of bovine gamma globulin alone and surviving late in the experiment (35 days) showed a similar incidence of lesions (70 per cent). However, valvular alterations were not as frequent in animals treated with bovine albumin alone (incidence of 22 per cent).

4. Subendothelial Leucocytic Infiltration of the Ascending Aorta and Pulmonary Arteries. These lesions were similar to those in previous experiments (4, 15). They are recorded separately in order to distinguish them from the more severe forms of arteritis.

5. Arteritis. Thirty-five per cent of 17 animals dying within 19 days while receiving multiple injections of both albumin and globulin showed marked acute necrotizing arteritis similar to that observed after a single intravenous

injection of albumin alone (4). This lesion consisted of necrosis of one or more layers of the vessel wall accompanied by polymorphonuclear and mononuclear cellular infiltration (Figs. 1, 2). No healing or healed lesions were observed in this group.

The incidence of arterial lesions was much greater in animals receiving antigen for longer periods of time. Further, in animals surviving beyond the 19th day, healing and healed arterial lesions were also encountered (Figs. 5–8). These were observed in animals receiving albumin or globulin or both. Healing lesions showed early intimal fibrosis and infiltration of the adventitia, and to a lesser degree, other arterial coats by lymphocytes (Figs. 5–7). Healed altera-

TABLE VI

Summary of Incidence and Distribution of Arterial Lesions

Antigen	Time of Death or Sacrifice	No. of Rabbits	Per cent of animals with			Total arteritis	Per cent of animals with following no. of organs showing arterial lesions					
			Acute or partially healed necrotizing (active) arteritis only	Healing or healed arteritis* only	Both necrotizing and healing or healed arteritis		1	2	3	4	5	6
Both bovine albumin and gamma globulin	7–19 days; Av. = 12 days	17	41	0	0	41	18	12	12			
	22–48 days; Av. = 34 days	21	19	33	33	86	33	10	10	24		10
Bovine albumin only	35 days	9	11	44	22	78	22	11	44			
Bovine gamma globulin only	34–35 days	10	30	40	0	70	40		30			

* Without obvious necrosis.

tions consisted of marked narrowing of the lumen by fibrous thickening of the intima, more or less replacement of the media by fibrous tissue, periarterial fibrosis, and the absence of inflammatory cells (Fig. 8). Acute arteritis was not always present in animals showing healing or healed arterial lesions. However, 33 per cent of the animals receiving both albumin and globulin and surviving late in the experiment showed both acute and healing or healed lesions. The incidence and distribution of the arterial lesions of various ages are recorded in Table VI.

As with a single injection of antigen (1, 2, 4), the vessels of the heart and mesentery were frequently affected. Arterial lesions in the liver, renal hilum, stomach, intestine and bladder were less constantly observed. The widest distribution of arterial lesions was seen in animals receiving both albumin and globulin from periods ranging from 19 to 48 days.

No arterial lesions were present in the kidneys of animals dying early in the experiment. This finding is in agreement with previous observations on the

failure of single injection of antigen to induce renal arterial lesions (4). However, the intrarenal arteries were commonly affected in the group of animals treated for a prolonged period with combined antigens. In the kidneys of 6 of 21 animals (29 per cent) there was widespread necrotizing and granulomatous arteritis involving the arcuate and interlobular arteries (Figs. 9–15). The necrotizing arteritis was similar to that occurring in other organs (Figs. 10, 11). The granulomatous arteritis was observed only in the kidney; this lesion

TABLE VII

Character and Frequency of Renal Arterial Lesions

Rabbit No.	Treatment	Number of Arteries Showing Following Types of Inflammation‖					Total‡
		Necrotizing	Granulomatous with necrosis	Granulomatous without necrosis	Perivascular plasma cells	None	
961	Bovine Albumin Only	4(a)†	0	0	9(a)	2(a)	5/51 (10%)
		1(i)			26(i)	9(i)	
949		4(a)	2(a)	0	12(a)	4(a)	6/62 (10%)
					19(i)	21(i)	
946	Bovine Albumin and	2(a)	7(a)	0	7(a)		9/16 (56%)*
354	Bovine Gamma Globulin	0	10(a)	2(a)	1(a)	4(a)	32/56 (57%)
			3(i)	17(i)	17(i)	2(i)	
356		3(a)	8(a)	1(a)	2(a)	5(a)	41/57 (72%)
		5(i)	16(i)	8(i)	4(i)	5(i)	
912		0	2(a)	3(i)	**	57	5/61 (8%)
932		0	1(i)	4(a)	13(a)	1(a)	28/65 (43%)
				23(i)	22(i)	1(i)	
346		0	0	2(a)	8(a)	7(a)	20/60 (33%)
				18(i)	21(i)	4(i)	

* Represents per cent of arcuate arteries with lesions; does not include interlobular arteries.

** Not enumerated because of pyelonephritis.

‖ One section of kidney examined.

† a = arcuate; i = interlobular.

‡ Numerator = total number of arteries with lesions; denominator = total number of arteries in kidney section. Arteries with surrounding plasma cells are not included as lesions.

consisted of extensive replacement of the media of the small renal arteries by infiltrates of macrophages and epithelioid cells (Figs. 9, 12–15). Often, the arterial lumen was more or less completely obliterated and only the presence of an arterial segment coming to and from the lesion revealed its vascular nature. In such instances the granulomatous arteritis closely simulated an "epithelioid" tubercle of tuberculous infection (Figs. 13–15). The granulomatous vascular lesions were invariably surrounded by abundant plasma cells. Plasma cells were often seen in profusion about normally appearing arteries and about unaltered glomeruli. It is of interest that the intrarenal

arteritis was present only in animals showing widespread arteritis of other organs (Table IV). Two animals receiving albumin alone showed intrarenal arterial lesions. One of these contained poorly developed granulomatous arteritis. The frequency and distribution of the renal arterial lesions are recorded in Table VII.

B. Immunologic Findings

Since additional antigen was injected only when the previous dose had disappeared from the circulation, the number of injections of antigen which a given animal received was proportional to its ability to eliminate the antigen; those which eliminated the antigen most rapidly received the greatest number of injections. The number of injections of bovine albumin in animals receiving this antigen alone for a 35 day period varied from 4 to 13, with a median of 10. Thus, the average animal received an injection of 0.5 gm. of bovine albumin every 3 to 4 days, or a total of approximately 5 gm. During a similar time interval, animals treated with 2 gm. of bovine gamma globulin required a further injection on the average every 6 days or a total of approximately 12 gm. of this antigen. Animals receiving both albumin and globulin required amounts of each comparable to those in the single antigen groups.

Since the rate of antigen elimination is largely dependent on the immune response, an absence or paucity of allergic lesions might be anticipated in animals requiring few injections of antigen. Thus, animals 931 (albumin only), 925 and 955 (globulin only) and 347, 928, and 967 (both antigens) which utilized the smallest amount of antigen showed either trivial lesions or none at all. However, in animals with well-marked lesions, there was no clear-cut relationship between the severity of allergic lesions and the magnitude of the immune response as measured by the frequency of antigen injections. A marked individual variation in the severity of allergic lesions in animals with apparently similar immune responses has been noted in a previous study (4), indicating that there are factors other than the immune response which are active in determining the development of lesions.

DISCUSSION

The allergic lesions encountered following a single injection of antigen undergo a progressive orderly change from acute necrotizing and inflammatory to healing and healed stages. This histologic sequence has been clearly demonstrated following single injections of bovine albumin and injections of bovine gamma globulin (2, 4, 17, 18). The lesions developed during the immune phase of antigen elimination (while antigen-antibody combination is occurring) and regressed after free antigen had been eliminated and free antibody had appeared in the circulation. The time of development and duration of allergic lesions is therefore largely dependent on the rate of antigen catabolism and rate of antibody response in the presence of antigen (2, 4, 14, 17, 18).

The simultaneous occurrence of both acute and healed arterial lesions has rarely been noted following a single injection of purified antigen. Such a condition has occurred occasionally (1) but not regularly (9) following a single injection of a mixture of antigens, such as horse serum. However, in this case, the simultaneous occurrence of different stages of lesions is made possible because of successive immune or allergic responses to the different antigens contained in the serum. As shown by the present data, the simultaneous occurrence of both fresh and healed arterial lesions requires repeated antigenic insults to the body.

In addition to producing arterial lesions in different stages of development, in the same animal, the chronic administration of antigen led to a wider distribution of arterial lesions. Following a short period of sensitization, arterial lesions are most frequently encountered in the heart and mesentery (1–5). The great frequency with which the coronary arteries are damaged in experimental allergy has led some investigators to compare the arterial lesions to rheumatic arteritis rather than to human periarteritis nodosa (19). Lesions of the intrarenal arteries have been rarely noted following a single injection of antigen (1–4). However, intrarenal lesions were frequently observed in the present experiment in animals receiving both albumin and globulin for periods ranging from 19 to 48 days. Widespread damage to the renal vessels is of course common in human periarteritis nodosa.

The presence of granulomatous type arteritis (in this instance confined to the kidneys) in animals receiving prolonged sensitization is noteworthy. Granulomatous (tuberculoid) lesions, usually in or adjacent to the walls of affected arteries, are not unusual in human periarteritis nodosa (20). Occasionally such lesions may dominate the pathologic picture. Striking granulomatous arterial lesions have been observed in periarteritis nodosa produced by allergy to iodine, dilantin sodium, neoarsphenamine and sulfonamides (20–22). In allergic asthmatic granulomatosis (23) and in Wegener's syndrome (24–26) similar lesions are common in the respiratory system. In these conditions, the granulomatous lesions are always associated with the usual forms of periarteritis nodosa in numerous organs throughout the body. The occurrence of granulomatous lesions in the spleen and lymph nodes of rabbits receiving one or two injections of horse serum (20) or a single injection of bovine albumin (4) has been previously reported. Although in most instances such lesions are found diffusely throughout the lymphoid tissue, unrelated to large vessels, occasionally they are limited to the perivascular connective tissue and appear as granulomatous periarteritis. The present demonstration of the production of well-marked granulomatous arteritis in the kidney following the prolonged administration of antigen provides additional experimental evidence for an allergic etiology of cases of periarteritis nodosa exhibiting this type of lesion.

Although repeated injections of antigen led to the progressive development

of arterial lesions, the glomerular and splenic lesions, which were present following the first several injections of antigen, were not observed after continued sensitization. The reason for the failure of glomerular and splenic lesions to be continually produced by repeated large injections of bovine gamma globulin and bovine albumin, given alone or together, is not clear. A similar result was obtained in a previous study in which multiple injections of bovine gamma globulin alone were employed (16). On the other hand, McLean and his colleagues (12) have shown that daily injections of small quantities of horse serum given over many months may lead to marked glomerulonephritis in the absence of any discernible arterial changes. It is possible that the different results obtained by McLean and his workers were due to the different antigen dosage and method of antigen administration.

TABLE VIII

Effect of Length of Sensitization on the Character and Distribution of Allergic Lesions in Rabbits

	Brief Period of Sensitization (Single injection of antigen or multiple injections up to 3 weeks.)	Prolonged Period of Sensitization (Multiple injections of antigen for periods of 3 to 7 weeks.)
Arterial Lesions:		
Stage of Development	Acute (followed by healing and healed)	Acute, healing and healed
Character of Active Lesions	Inflammatory and necrotizing	Inflammatory, necrotizing, granulomatous
Distribution	Heart, lungs, mesentery	More widespread with extensive involvement of kidneys
Glomerular Lesions:	High incidence of acute proliferative and necrotizing lesions	No prominent lesions; occasional glomerular crescents, adhesions and scars
Splenic Lesions:	High incidence of granulomatous lesions in Malpighian bodies	No prominent lesions

The results of the present experiment, outlined in Table VIII, demonstrate the marked influence the length of antigen exposure may have on the character and distribution of allergic lesions. With brief periods of sensitization, damage to the entire vascular system, including the endocardium, arteries and capillaries, is usually evident, as indicated by a high incidence of valvulitis, acute necrotizing arteritis, proliferative and necrotizing glomerulonephritis and granulomatous splenic lesions. On the other hand, with prolonged sensitization produced by multiple large injections of antigen, the glomerular and splenic lesions tend to resolve, and widespread arteritis, consisting of acute and healing lesions of either the necrotizing or granulomatous type, dominates the pathologic picture.

The different types of lesions produced by experimental allergy have been compared to human periarteritis nodosa, glomerulonephritis and rheumatic

fever. However, all of the lesions produced experimentally in the rabbit can be observed in human periarteritis nodosa alone. Further, there is reason to believe that as in experimental allergy, the occurrence of a given type of lesion in human periarteritis nodosa may be influenced by the length of sensitization. Ball and Davson (27) have analyzed 16 cases of periarteritis nodosa with respect to the occurrence of splenic and glomerular lesions. A review of their data show that of 7 cases of periarteritis nodosa exhibiting only acute arterial lesions, all showed the presence of necrotizing glomerulonephritis and 5 of the 7 also contained splenic lesions. However, in the 9 cases showing healing and healed arterial lesions, indicative of a more prolonged disease state, glomerular and splenic lesions were present in only 2 instances.

The varied morphology of human periarteritis nodosa led Zeek and her colleagues (28–30) to adopt a classification consisting of "hypersensitivity angiitis" (equivalent to acute periarteritis nodosa with glomerular and splenic lesions), "allergic granulomatous arteritis" (where granulomatous vascular lesions are conspicuous), and "classical periarteritis nodosa" (where the only alterations encountered are arterial lesions in different stages of development). While attributing acute angiitis and granulomatous angiitis to allergy, Zeek and co-workers expressed the belief that hypertension rather than allergy might be the important factor in the pathogenesis of "classical periarteritis nodosa". However the morphologic differences which Zeek noted between hypersensitivity "angiitis" and "classical periarteritis nodosa" are not unlike those observed in the present experiment between animals acutely and chronically exposed to antigen.

While the groups of animals acutely and chronically exposed to antigen showed remarkable differences, it is important to point out that there was considerable variation in occurrence or extent of a particular lesion among the individual animals in each group. A similar situation is found in human periarteritis nodosa. The factors responsible for this marked individual variation are as yet unknown.

SUMMARY

1. The character and distribution of allergic lesions can be markedly influenced by the length of antigen administration. Following brief periods of sensitization, acute arterial lesions are found together with proliferative and necrotizing glomerulonephritis, endocarditis and splenic lesions. Following prolonged periods of sensitization produced by multiple large injections of antigen, acute, healing and healed arteritis occurs alone in the absence of glomerular and splenic lesions.

2. Prolonged sensitization leads to a high incidence of arterial lesions in the kidneys, many of which are granulomatous in character. The development of granulomatous arteritis in the kidney following repeated antigenic insults

supports the belief that granulomatous arteritis can result from protracted allergy.

3. The bearing of these results on the pathogenesis of the different morphologic variants of human periarteritis nodosa is discussed.

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EXPLANATION OF ILLUSTRATIONS

All photographs are of lesions encountered in animals receiving both bovine albumin and bovine gamma globulin.

FIG. 1. Rabbit 936, dead on 10th day after receiving 2 injections of albumin and 1 of gamma globulin. Advanced acute necrotizing coronary arteritis. Hematoxylin and eosin. ×100.

FIG. 2. Rabbit 355, dead on 12th day after receiving 2 injections of albumin and 3 of gamma globulin. Acute necrotizing coronary arteritis with early cellular reaction. Hematoxylin and eosin. ×125.

FIG. 3. Rabbit 936, dead on 10th day after receiving 2 injections of albumin and 1 of gamma globulin. Proliferative and focal necrotizing glomerulonephritis. Hematoxylin and eosin. ×175.

FIG. 4. Rabbit 952, dead on 10th day after 2 injections of both albumin and gamma globulin. Granulomatous reaction consisting of epithelioid cells, in splenic follicle. Hematoxylin and eosin. ×150.

FIG. 5. Rabbit 915, dead on 24th day after 5 injections of albumin and 5 of gamma globulin. Partially healed segmental necrotizing arteritis. Note intimal proliferation. Mesenteric lymph node. Hematoxylin and eosin. ×150.

FIG. 6. Rabbit 938, dead on 22nd day after 3 injections of albumin and 3 of gamma globulin. Partially healed necrotizing coronary arteritis. Note marked intimal and adventitial proliferation. Hematoxylin and eosin. ×150.

FIG. 7. Rabbit 912, sacrificed on 35th day after 6 injections of albumin and 4 of globulin. Partially healed necrotizing mesenteric arteritis. Hematoxylin and eosin. ×75.

FIG. 8. Rabbit 340, dead on 42nd day after 4 injections of albumin and 4 of globulin. Healed necrotizing mesenteric arteritis. Note remnants of circular smooth muscle of media and stellate fibroblasts in media and thickened intima. Hematoxylin and eosin. ×100.

FIG. 9. Rabbit 356, dead on 30th day after receiving 3 injections of albumin and 5 of globulin. Low power of kidney with widespread partially healed necrotizing and granulomatous inflammation of the arcuate and interlobular arteries. Hematoxylin and eosin. ×30.

FIG. 10. Rabbit 356, as before. Necrotizing renal interlobular arteritis with mononuclear cellular reaction. Many of the cells about the artery are plasma cells. Hematoxylin and eosin. ×250.

FIG. 11. Rabbit 356, as before. Necrotizing renal arcuate arteritis with slight granulomatous cellular reaction and numerous surrounding lymphocytes and plasma cells. Hematoxylin and eosin. ×250.

FIG. 12. Rabbit 932, sacrificed on 35th day after 6 injections of albumin and 4 of globulin. Granulomatous inflammation of a segment of renal interlobular artery. Hematoxylin and eosin. ×250.

FIG. 13. Rabbit 932, as before. Marked granulomatous reaction in and about small interlobular artery. Cellular reaction consists of nest of epithelioid cells surrounded by lymphocytes and plasma cells. Hematoxylin and eosin. ×300.

FIG. 14. Rabbit 932, as before. Granulomatous renal interlobular arteritis. Note tuberculoid nature of lesion. An uninvolved segment of artery can be seen in the left upper corner. Hematoxylin and eosin. ×300.

FIG. 15. Rabbit 932, as before. Granulomatous renal arteritis. Except for the presence of a small arterial lumen, this lesion closely resembles a tubercle of infection. Hematoxylin and eosin. ×400.

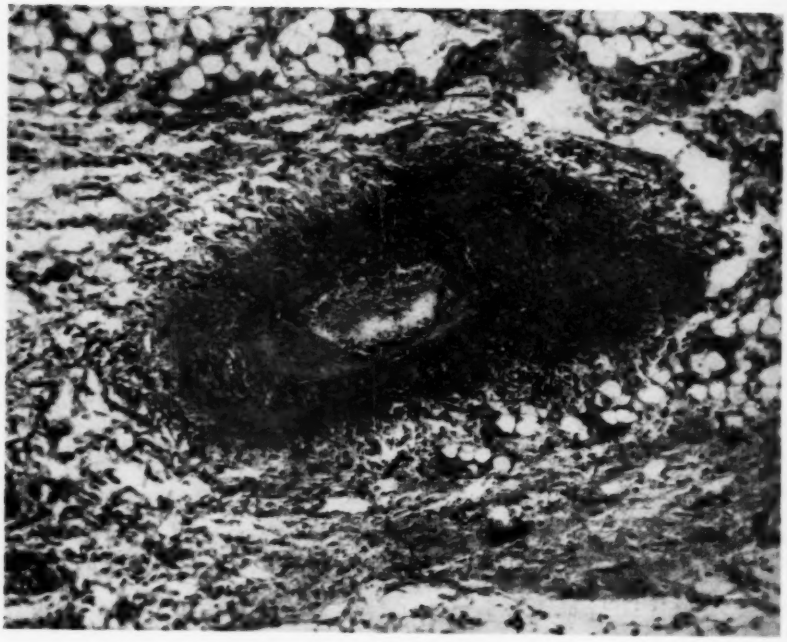
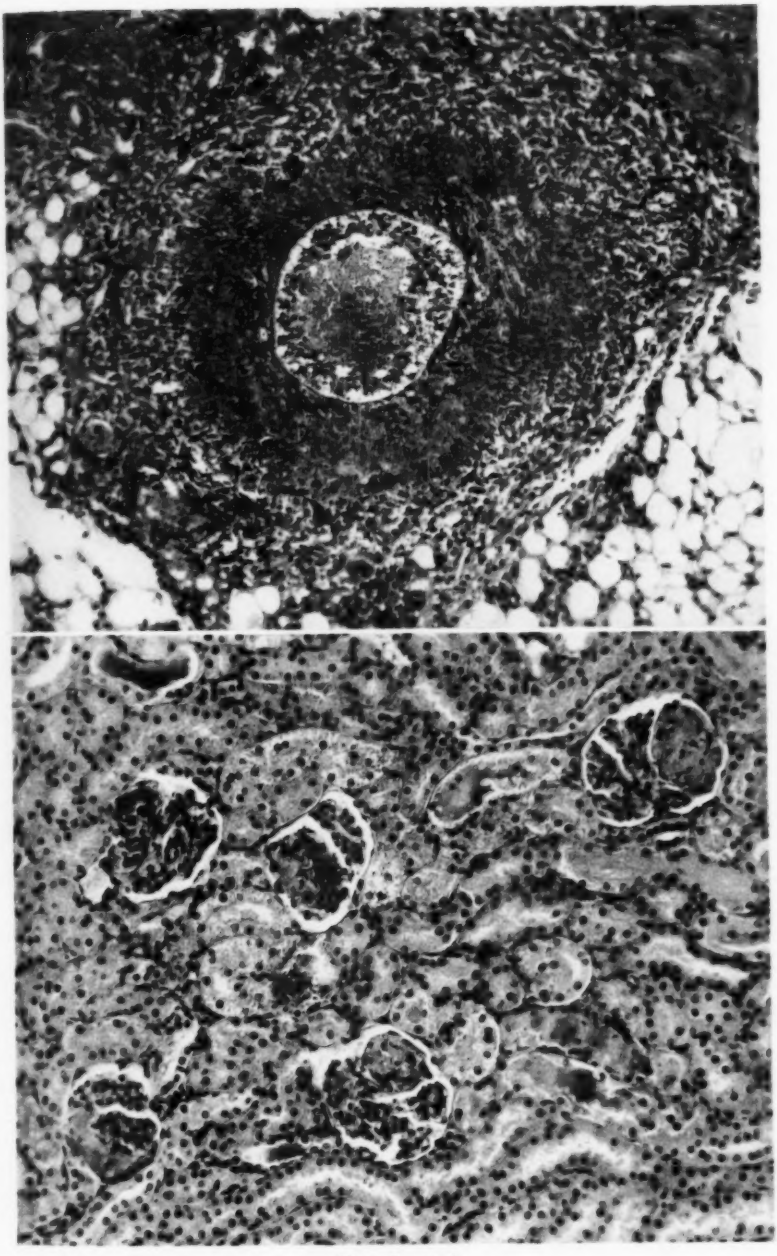
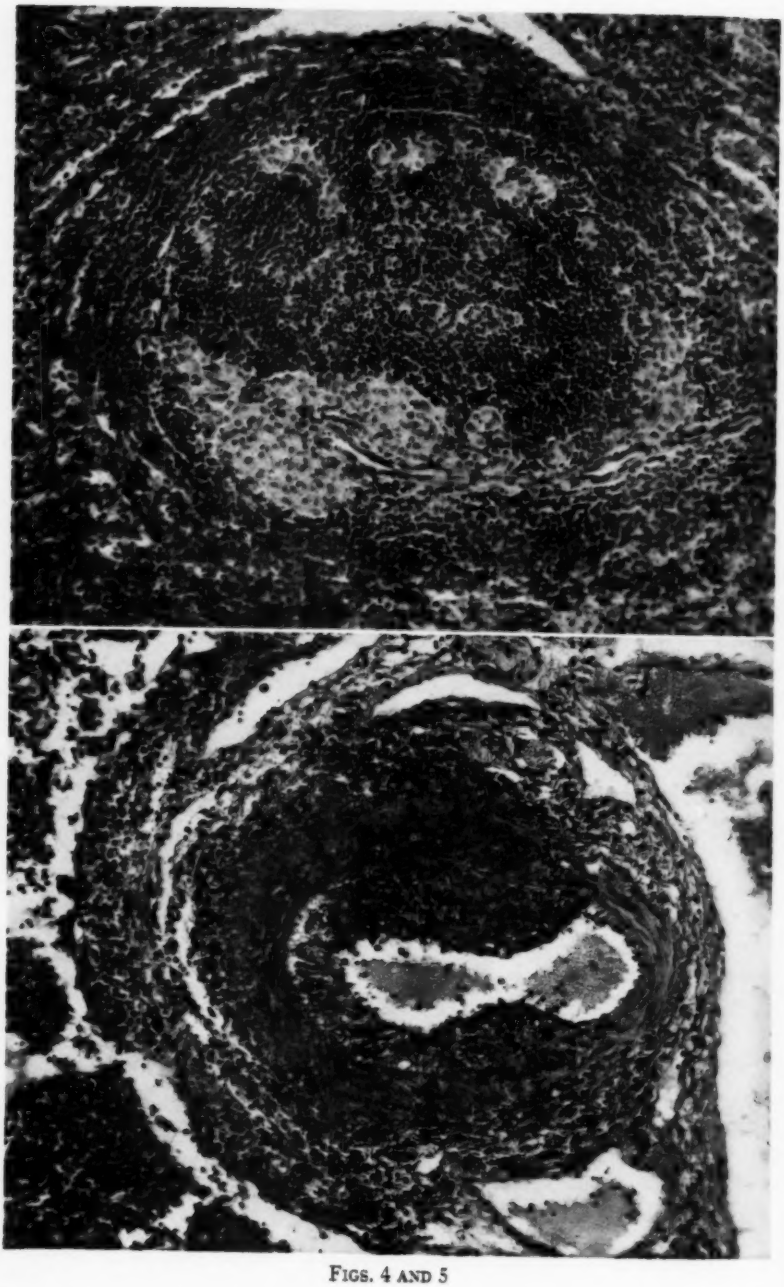


FIG. 1



FIGS. 2 AND 3



FIGS. 4 AND 5

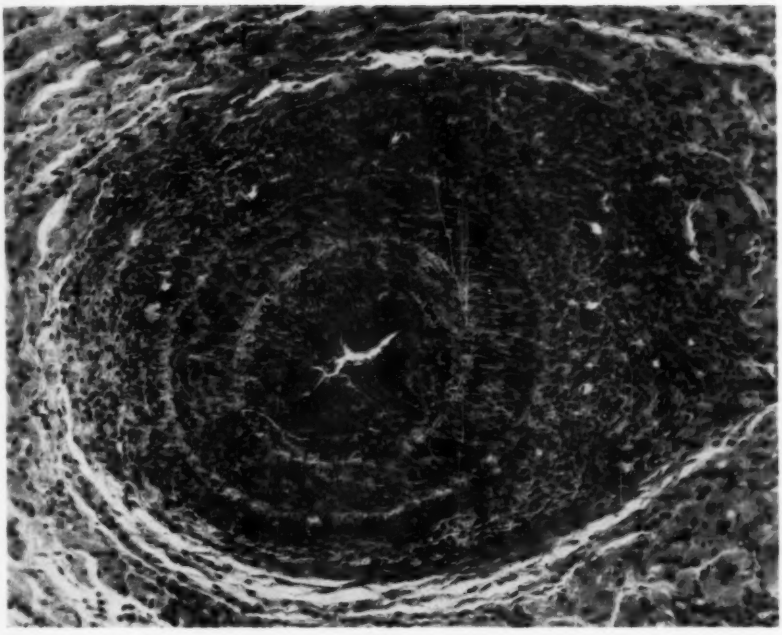
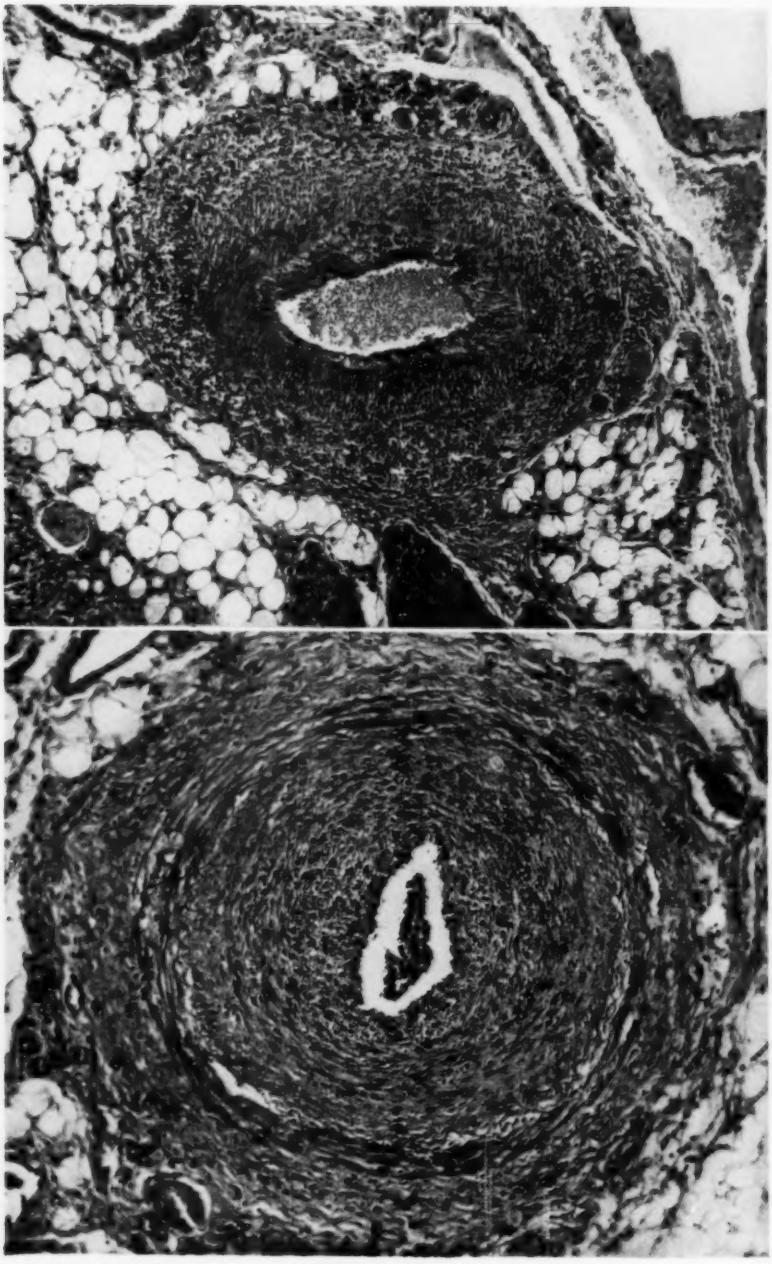
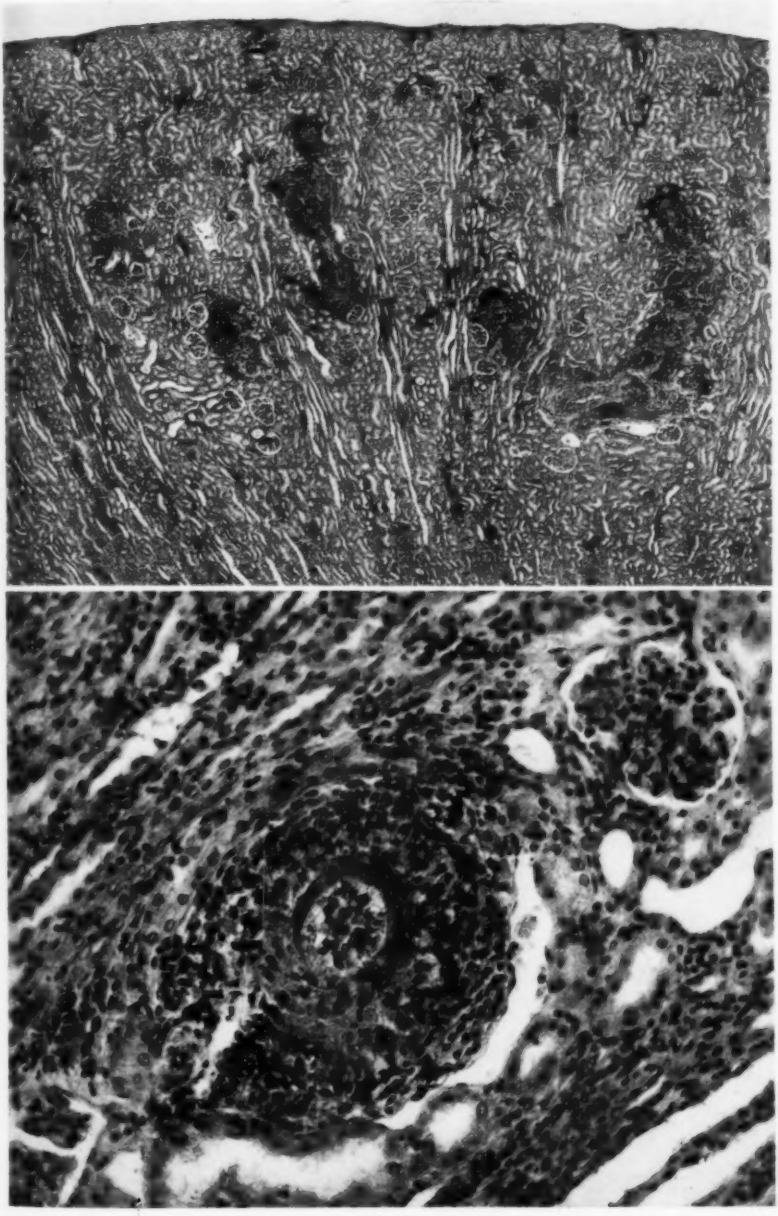


FIG. 6



FIGS. 7 AND 8



FIGS. 9 AND 10

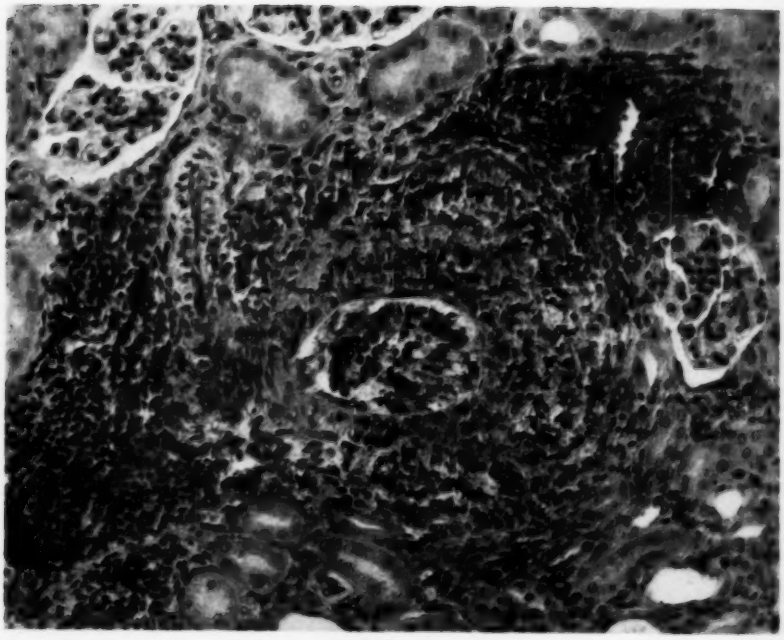
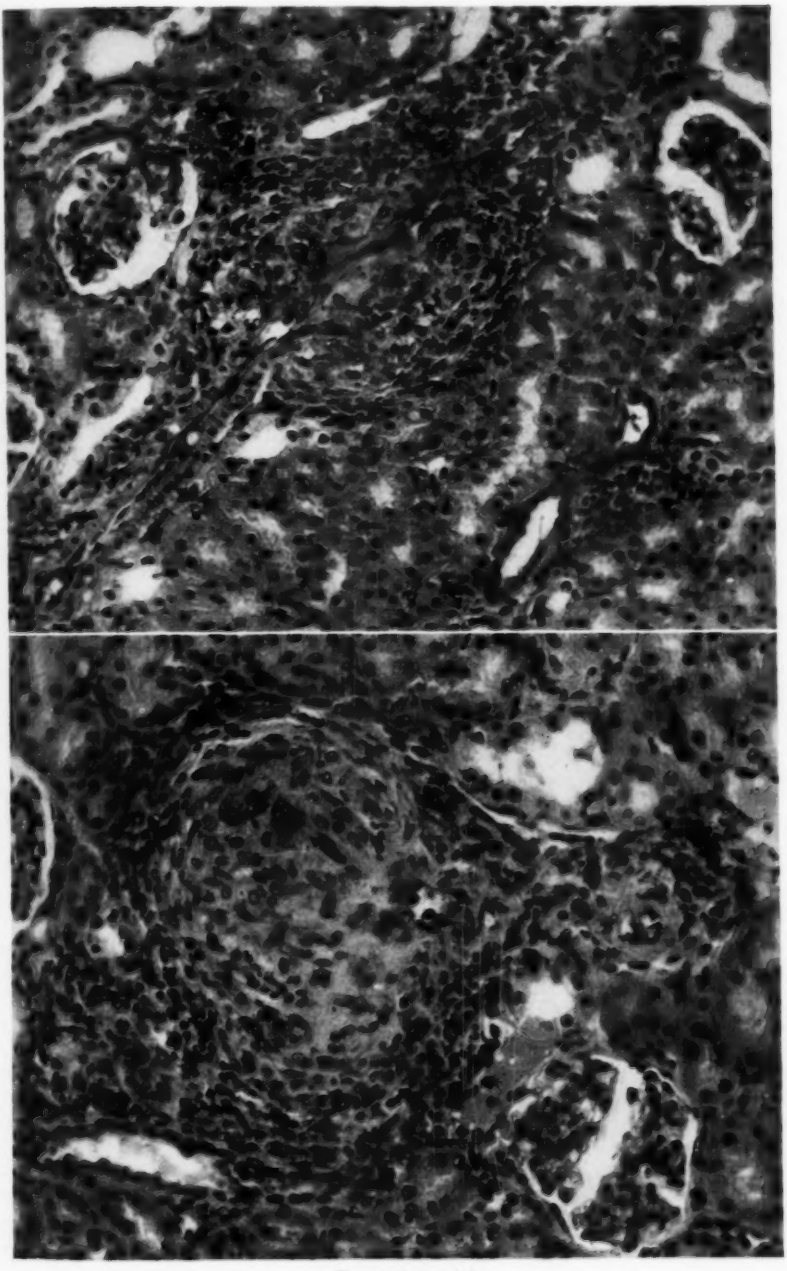
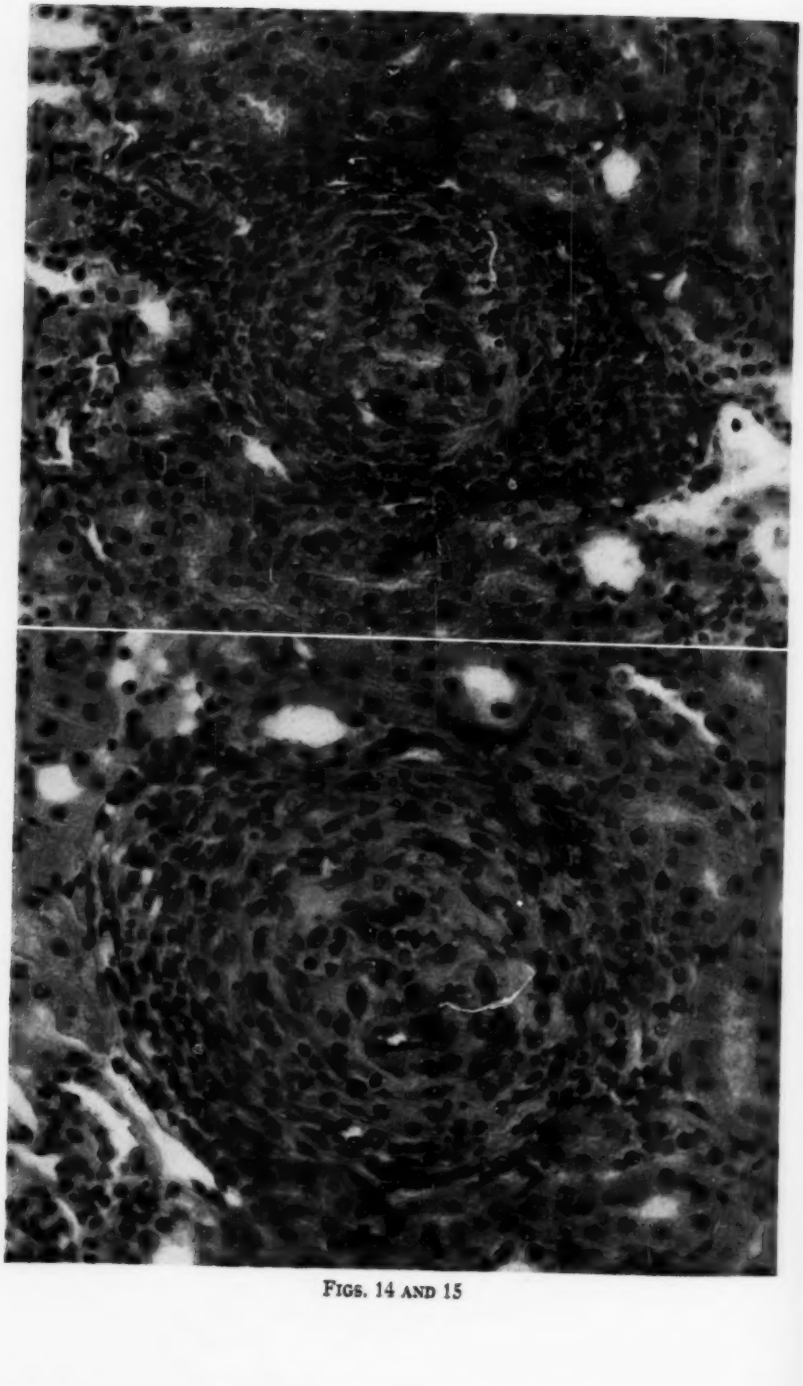


FIG. 11



FIGS. 12 AND 13



FIGS. 14 AND 15

ABSTRACT OF PAPER PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

November 12, 1955

CAPILLARY PERMEABILITY IN CONGESTIVE HEART FAILURE

RICHARD S. ROSS AND W. GORDON WALKER

The relationship between the masses of protein in the vascular and extravascular spaces and the transcapillary exchange rate of protein has been investigated in normal individuals and in individuals with congestive heart failure. Plasma disappearance curves of I^{131} labelled human serum albumin have been constructed from observations extending over a fourteen day period following a single intravenous injection. The isotope dilution principle has been utilized to estimate the size of the vascular and extravascular protein masses.

From the first portion of the plasma disappearance curve a "mean transcapillary exchange rate" is derived. This portion of the curve is presumably due to the equilibration of vascular protein with the extravascular protein mass during the first three to four days after injection. In two experiments, measurements made on Southey tube drainage fluid validate this assumption that the first portion of the curve represents equilibration between vascular and extravascular protein masses.

In eleven normal individuals the transcapillary exchange rate is more closely correlated with the mass of circulating protein ($r = +.7$; $p < .001$) and with the plasma volume ($r = +.6$; $p < .02$) than with the serum protein concentration. A plot of this data reveals that the "mean transcapillary exchange rate" rises with increasing plasma volume and increasing protein mass. When the regression line of plasma volume on exchange rate is extrapolated to the ordinate, the intercept is significantly different from zero, suggesting that no transcapillary exchange of albumin occurs at plasma volumes below 20 ml./kg. of body weight. A similar plot of exchange rate and vascular albumin mass reveals an intercept on the ordinate at 0.6 gm. of circulating albumin per kg. of body weight. These observations suggest that the quantity of protein in the extravascular pool is governed by the permeability of the capillaries and that there exists a level of vascular protein mass below which no transcapillary protein exchange takes place. A plot of vascular albumin versus extravascular albumin points up the same relationship in that extravascular albumin mass falls to zero as vascular albumin approaches 0.6 gm./kg. In order to test this concept further, plasma disappearance curves were obtained in two patients with marked hypoproteinemia resulting from the nephrotic syndrome. The quantity of extravascular albumin was quite small in one of these individuals and virtually non-existent in the other. Thus it appears that changing capillary permeability serves to maintain plasma volume within relatively narrow limits despite wide variation in the total mass of exchangeable albumin. At low levels of plasma volume, below 20 ml./kg., transcapillary protein exchange appears to cease, and as plasma volume rises the rate of transfer of protein to the extravascular pool is increased. In the group of normal individuals an eight-fold variation in total exchangeable albumin is accompanied by only a three-fold change in plasma volume. There are two possible explanations for this change in permeability with changing vascular protein mass: First, that there is an increase in the permeability of the capillary membrane while the total area available for exchange remains constant; and, second, that the available area increases with the permeability per unit are a remaining constant. The second of these possible explanations seems unlikely since it implies that there are no open capillaries at the low levels of plasma volume at which exchange appears to cease. It is hoped that experiments employing two labelled proteins

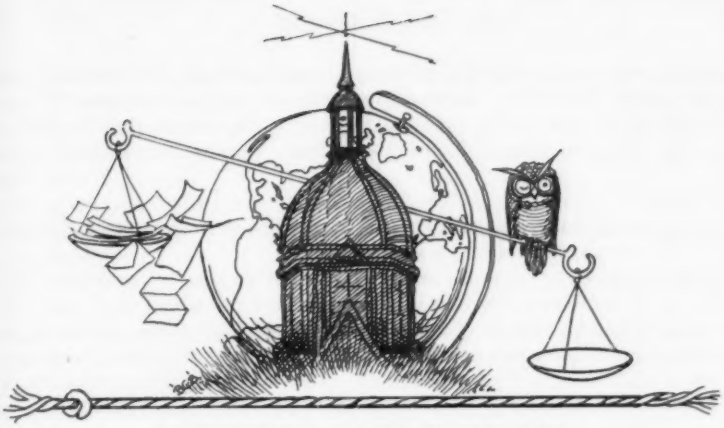
of different size will give information regarding the mechanism whereby capillary permeability is changed.

In sharp contrast to the relations seen in the normal subjects are the findings in ten patients with congestive heart failure. In this group of individuals the relationship between "mean transcapillary exchange rate" and plasma volume is reversed ($r = -.6$). The larger the plasma volume, the smaller the exchange rate. It would appear that this alteration in the normal relationships may account for the increase in plasma volume known to be characteristic of congestive heart failure. As a natural consequence of this decreased permeability of the capillaries to protein there is a significant decrease in the fraction of the total exchangeable protein which is extravascular. The decrease in the "mean transcapillary exchange rate" on heart failure could be the result of a change in the characteristics of the membrane, a change in the area available for exchange, or a redistribution of the blood volume so that less blood is in the more permeable capillaries. For many years increased capillary permeability has been suggested as a possible factor in edema formation in congestive heart failure, but the present investigations indicate that permeability, at least to protein molecules, is decreased in this disease.

BIOLOGICAL AND MEDICAL RESEARCH IN THE ARCTIC, WITH A FILM ON THE LIFE HISTORY OF THE ADELIE PENGUIN

W. J. L. Sladen

Oxford University



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Polysaccharides in Biology. Transactions of the First Conference, April 27, 28 and 29, 1955. Edited by GEORG F. SPRINGER. 271 pp., $5.00. The Josiah Macy, Jr. Foundation, New York, N. Y.

This slim volume presents what appears to be an actual transcript of the presentations and discussions at a multiprofessional conference dealing with polysaccharides of biological interest. The topics taken up were (1) Problems of Communication: Nomenclature, M. L. Wolfrom; (2) Problems of Classification, Karl Meyer; (3) Bacterial Polysaccharides, Michael Heidelberger; (4) Blood Group Substances, W. T. J. Morgan.

In keeping with the informal atmosphere of the conference, presentations were frequently interrupted. In this book, comments are recorded as they occurred; to this reader it was sometimes annoying to be taken from the line of thought of the discussor to pursue tangential considerations.

With this reservation, those interested in polysaccharides of biologic significance will find sections 3) and 4) of particular value. Sections 1) and 2) serve primarily to emphasize that there is still no uniform classification or really useful nomenclature.

PAUL D. HOEPRICH

Clinical Examinations in Neurology. By Members of the Sections of Neurology and Section of Physiology, Mayo Clinic and Mayo Foundation for Medical Education and Research School, University of Minnesota, Rochester, Minn. 370 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.

When a group of competent men sets out to put down on paper a description of methods, the result is bound to be informative. That is true of the present book. The members of the Sections of Neurology and Physiology at the Mayo Clinic have, in tribute to their mentors Doctors Henry Woltman and Frederick Moersch, described the methods and records of neurological examination which are in use at that center.

The book was designed as a descriptive manual to help the Fellows in training there in "mastery of the clinical neurological examination." As such, it no doubt serves a very useful purpose. Despite its inevitable rigidity, local colour, and lack of criticism, it should also be

of value to residents in training elsewhere as a ready reference. Considerable descriptive material, already existent from other sources, is brought together in one place. It is the impression of the reviewer however, and acknowledged by the authors, that the utility of the book is limited to that purpose. It would be of limited value in undergraduate teaching.

J. W. MAGLADERY

Meditations on Medicine and Medical Education Past and Present. By I. SNAPPER. 138 pp., $3.75. Grune & Stratton, New York, N. Y.

The book is divided into three sections: Physicians and Surgeons in New Netherland Under the Dutch, The Influence of Boerhaave of Leyden on Medicine in the U. S. A., and The Post-Boerhaavian Centuries. The author's knowledge of Dutch sources (he was born in Amsterdam where for many years he served as Professor of Medicine and General Pathology at the University) allows him to utilize material that is not easily accessible to others. While the bulk of the book is historical in nature, in the final pages, Dr. Snapper utilizes the results of the second and third sections for his "meditations" on past and present conditions. Briefly speaking, he thinks that Hippocrates represented the right combination of clinical empiricism and scientific research, and that his method was revived by Hermann Boerhaave whose teaching, via Leyden and Edinburgh, influenced American medicine. He believes that in our own days, there is danger of neglect of sound clinical teaching in favor of a one-sided scientific approach (as once in Knidos) and delegation of ward instruction to young men with little experience. Much of the historical material presented by the author is of great interest, though the Hippocrates he depicts appears somewhat of a symbolic figure. Dr. Snapper's ideas about the advantages of combining the best in American and Continental European teaching methods, and his plea for freeing professors from administrative duties, so that they can devote themselves more completely to clinical instruction and research, deserve an attentive hearing.

OWSEI TEMKIN

Group Processes. Transactions of the Second Conference October 9, 10, 11 and 12, 1955. Edited by BERTRAM SCHAFFNER. 255 pp., $3.50. The Josiah Macy, Jr. Foundation, New York, N. Y.

This small but stimulating volume presents the proceedings of the second of a series of five conferences on "Group Processes" sponsored by the Josiah Macy, Jr. Foundation. The chief theme of this conference is the nature and meaning of "play." In contrast to the first report of this series, the discussions seem to have been helpfully arranged and edited, so that the reader often finds himself in the thick of the conference as a vicariously participating member.

The book begins with brief autobiographical sketches of the discussants which set the frame of reference for their contributions which follow. The major presentations about which the conference was organized are: Social Structure among Penguins by W. J. L. Sladen; Neonate-Mother Relationship in Goat and Man by H. Blauvelt; Kinesic Analysis of Filmed Behavior of Children by R. L. Birdwhistle; and The Message "This is Play" by G. Bateson. The discussions resulting from the last cited topic are fascinating to say the least. A variety of viewpoints concerning play and its role in growth, relationships and living in general are developed. To a large extent, these delineate what play is not. In fact, one of Bateson's definitions of play is that it is a class of behaviors delimited "by means of a negative without commonly identifying what that negative negates . . ." in the category "of improper nots presumably related but usually undefinably related to more central and positively defined categories." Interesting examples of human behavior, normal and pathological, and of infra-human behavior which bear on play and its meaning are presented not so

much for their value as factual information but as a means of provoking stimulating group discussion. Though this section is the most exciting in the entire book, especially to those with a philosophical and psychological inclination, this is not to minimize the value of the remaining discussions, all of which are absorbing demonstrations of creative thinking in action.

The book is highly recommended reading. If each of the succeeding conference reports improve as much as this over the previous one, they will be eagerly awaited and enthusiastically received.

LESTER H. GLIEDMAN

Shock and Circulatory Homeostasis. Transactions of the Fourth Conference December 6, 7 and 8, 1954. Edited by Harold D. Green. 291 pp., $5.00. The Josiah Macy, Jr. Foundation, New York, N. Y.

This issue of transactions of the Macy Conference on Shock and Circulatory Homeostasis deals more with the latter than the former subject. As in earlier issues the informal presentation of experimental work and observations by members of the conference with frequent interruptions for contributions of dissenting opinions, supplementary data and altered interpretations makes for delightful and informative reading. The result is similar to panel discussion and presentations which have become an important part of scientific meetings. Discussions are concerned with the action of epinephrine in man, the peripheral circulation with studies of flow and electrode estimation of oxygen tension, the splanchnic circulation, the general characteristics of the pulmonary circulation and a few changes in ventilation and flow which occur in this circuit with shock, and finally, recent studies of the coronary circulation and its intrinsic control. The volume is recommended to students of these topics.

HENRY T. BAHNSON

Treatment of Heart Disease. By HARRY GROSS AND ABRAHAM JEZER. 549 pp., $13.00. W. B. Saunders Co., Philadelphia, Pa.

The authors state in the preface that their aim is to provide a sound physiological basis for discussing the treatment of circulatory disease and they have done so with great clarity. Whoever learns therapy from this book will know precisely what he is trying to accomplish and why he is trying to do it.

Conventional subjects are dealt with comprehensively, in a thoroughly up to date manner and there are no important omissions. Several sections are particularly outstanding. Those dealing with congenital heart disease and surgery in the cardiac patient present many stimulating points of view.

A great deal of attention is given to psychological factors in the conduct of therapy and rehabilitation and even the experienced physician will find these sections of real practical value to him.

And finally, an appendix dealing with diets is especially valuable. With information provided here the physician can not only prescribe dietary restriction soundly but he can also do much to take away the sting of drastic food restriction.

The book is recommended with great enthusiasm.

BENJAMIN M. BAKER, JR.

Human Ovulation and Fertility. By EDMOND J. FARRIS. 159 pp., $6.50. J. B. Lippincott Co., Philadelphia, Pa.

Human Ovulation and Fertility by Edmond J. Farris, Ph.D., Executive Director of The Wistar Institute, is a resumé of the authors extensive experience with the rat ovarian hyperemia as a technique for the determination of ovulation. For clinicians or research scien-

tists interested in this rather specialized field the material and statistics, as well as the review of the literature, cannot fail to prove interesting. The vast clinical importance ascribed to the precise timing of ovulation detracts somewhat from the material as this premise is still highly debatable.

G. E. SEEGAR JONES

Pulmonary Carcinoma. Pathogenesis, Diagnosis and Treatment. Edited by EDGAR MAYER AND HERBERT C. MAIER. 540 pp., $15.00. J. B. Lippincott Co., Philadelphia, Pa.

This monograph is a thorough and complete compilation of current knowledge on the subject by the editors with sixteen other contributors, almost all from the several schools and centers of New York City. A satisfyingly aggressive approach is advocated throughout the many chapters which deal with all aspects of the disease from cancer research through pathology, diagnosis (with emphasis on diagnosis in the incipient, asymptomatic stage), surgery, radiation, drug and supportive therapy of the patient with an inoperable lesion, to the psychological aspects and with even a forecast of the outlook for cancer.

Almost all sections are of special and practical value. Pathology is well covered by Liebow and Papanicolaou, bronchoscopy and surgery by Maier, clinical and medical aspects by Mayer, LaDue, and Rappaport, and other facets by equally competent authorities in each of the special fields. The practical approach is carried through to the appendix which contains abstracts of 25 cases demonstrating "pitfalls in diagnosis and lessons learned in retrospect."

The editors are admirably successful in accomplishing their objective: "to produce a predominantly practical book written by clinicians and specialists reflecting for the most part their own views and opinions gleaned from an extensive clinical experience."

The increasing prevalence of the disease makes this book timely and important to all concerned with the care of patients and to them it is highly recommended.

HENRY T. BAHNSON

Diagnosis and Treatment of Peripheral Vascular Disorders. By DAVID I. ABRAMSON. 537 pp., $13.50. Paul B. Hoeber, Inc., Medical Book Department of Harper & Brothers, New York, N. Y.

The material in this volume is divided in an unusual manner. The three parts of the book are entitled: 1. Differential Diagnosis of Symptoms and Signs; 2. Peripheral Vascular Diseases; and, 3. Anatomic, Physiologic, Pharmacologic and Pathologic Considerations of the Peripheral Circulation. This order is appropriate in a book designed to serve as a "ready source of reference" for the physician who encounters peripheral vascular problems rarely in his practice. The first section includes many pages of tables listing signs, symptoms, and the results of simple tests in similar diseases. There is, for example, a six-page table outlining the salient diagnostic features of extremity pain in a wide variety of disorders. Such a table will be useful to the practitioner who needs a reference source but who lacks the time necessary to extract the same information from books organized by diseases.

In the second section, the diseases of the peripheral circulation are discussed individually. The concept of peripheral vascular disease exhibited in the second section is broad in that nineteen pages are devoted to the "collagen disorders". The suggested therapeutic programs for peripheral vascular diseases are apparently based largely on the author's personal experience. Little reference is made to clinical trials of various therapeutic agents and critical evaluation of therapy is often lacking. Some of the principles outlined are not generally accepted and no evidence for their efficacy is given. For example, great stress is laid on the local application of antibiotic preparations to leg ulcers, and the intra-arterial injection of

penicillin is suggested as a useful procedure in this condition. Routine parenteral or oral administration of antibiotics is considered of value only "as a supplementary measure." The third part of the book, dealing with physiological and pharmacological principles, gives a good but superficial background for the foregoing chapters. References to the original literature are infrequent, but in view of the primary purpose of the book they are probably unnecessary. In conclusion, the book can be recommended for the physician's office, where it will serve as a source of practical diagnostic and therapeutic information.

RICHARD S. ROSS

BOOKS RECEIVED FOR REVIEW

Administrative Medicine. Transactions of the Fourth Conference October 31, November 1 and 2, 1955. Edited by GEORGE S. STEVENSON. 251 pp., $4.25. The Josiah Macy, Jr. Foundation, New York, N. Y.

Aids to Medicine, 6th edition. By J. H. BRUCE. 383 pp., $3.00. Bailliere, Tindall & Cox, London, England. Distributed by The Williams & Wilkins Co., Baltimore, Md.

Fractures, Dislocations and Sprains, 6th edition. By JOHN ALBERT KEY AND H. EARLE CONWELL. 1168 pp., $20.00. The C. V. Mosby Co., St. Louis, Missouri.

Functions of Autonomic Transmitters. The Abraham Flexner Lecture Series Number Thirteen. By J. HAROLD BURN. 228 pp., $5.00. The Williams & Wilkins Co., Baltimore, Md.

Fundamentals of Immunology, 3rd edition, revised. By WILLIAM C. BOYD. 776 pp., $10.00. Interscience Publishers, Inc., New York, N. Y.

Glaucoma. Transactions of the First Conference December 5, 6 and 7, 1955. Edited by FRANK W. NEWELL. 251 pp., $4.50. The Josiah Macy, Jr. Foundation, New York, N. Y.

Handbook of Chest Surgery for Nurses. By J. LEIGH COLLIS in collaboration with L. E. MABBIT, 4th edition revised by J. LEIGH COLLIS. 196 pp. The Williams & Wilkins Co. Baltimore, Md.

The Merck Manual of Diagnosis and Therapy, 9th edition. Edited by CHARLES E. LYGHT. 1870 pp., $6.75. Merck & Co., Rahway, N. J.

The Person Behind the Disease. By JULIUS BAUER. 136 pp., $3.50. Grune & Stratton, Inc., New York, N. Y.

Progress in Nuclear Energy. Series VII. Medical Sciences. Edited by J. C. BUGHER, J. COURSAGET AND J. F. LOUTIT. 165 pp., $6.00. McGraw-Hill Book Co., New York, N. Y.

Sick Children. Diagnosis and Treatment, 7th edition. By DONALD PATERSON. Revised by REGINALD LIGHTWOOD with the assistance of F. S. W. BRIMBLECOMBE. 395 pp., $8.75. J. B. Lippincott Co., Philadelphia, Pa.

PUBLISHED ON JANUARY 30, 1957:

The Physiology of Nerve Cells

BY JOHN C. ECCLES

A foremost nerve specialist here gives an account of the most recent research which has been done on the physiology of nerve cells. This work has only been possible in the last few years depending, as it does, on very recent technical developments, particularly microelectrode recordings from the interior of nerve cells. Many of the ideas expressed in this book have never before been published, even in the specialized scientific journals.

The book begins with an account of the electrical properties of the nerve cell and goes on to show how various excitatory reactions of nerve cells can be accounted for in terms of this picture. For the first time, such fundamental events in nerve cell behavior as the synaptic excitatory reactions of the motoneurone and the inhibitory responses of nerve cells are explained in terms of changes of membrane permeability and the consequent movements of ions along electrochemical gradients.

A later part of the book is devoted to a study of the pathways in the central nervous system, in particular those concerned in the simpler kinds of inhibitory responses. The final chapter, more speculative in nature, attempts to develop ideas of the relationship between structure and function, not only of nerve cells and pathways, but also of the microstructure of the synapse and the electrical and chemical mechanism of its operation.

288 pages

80 illustrations

$5.75

the author

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the contents

I. The Nerve Cell and Its Surface Membrane
II. The Excitatory Reactions of Nerve Cells
III. The Inhibitory Reactions of Nerve Cells
IV. The Interaction of the Excitatory and Inhibitory Responses of Nerve Cells
V. Pathways and Synaptic Transmitter Substances in the Central Nervous System
VI. Aspects of the Functional Architecture of the Central Nervous System

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